TEXTBOOK OF BIOCHEMISTRY

Fifth Revised Edition

A.V.S.S. Rama Rao

Textbook of Biochemistry

For Medical Students

Fifth Revised Edition

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FOREWORD TO THE FIRST EDITION

Dr. A.V.S.S. RAMA RAO, the author of this textbook of biochemistry deserves to be congratulated on presenting to the medical student at different levels of education (undergraduate and postgraduate), specialist in biochemistry and to the physician (different specialities), comprehensive and clear account of the current principles of biochemical knowledge. This he has been able to do by drawing richly on his experience as a teacher in biochemistry in medical colleges for over fifteen years in more than one University. He has also been able to put through in the book, as a critical thinker, the quantum of knowledge that is to be imparted in the evergrowing speciality – biochemistry. He has been able to do so owing to his exposure to the trends in medical education in basic sciences at the Harvard School of Medicine and a dozen more medical schools in the U.S.A.

He has, in this book, defined the grammar of biochemistry, outlined the historical developments in the subject and made the presentation in a narrative form, so that it is made palatable to the consumer–the medical student, biologist and investigator. In this Dr. Rao has adopted the conventional and traditional method of characterizing the food constituents, describing their formulae, their properties and their place in human biological and chemical reactions. The language is simple and easily understandable.

The author has rightly stressed the need for the medical student to posses a thorough knowledge of inorganic, organic and physical chemistry as the basis and pre-requisite for a study of biochemistry. The historical developments in the knowledge of biochemistry and its applications to clinical medicine are traced from the fifteenth century to the modern times. In some chapters, the author pertinently gives descriptive account of the historical evolution of the subject matter as an introduction. The chapters on metabolism of carbohydrates, lipids, proteins, nucleic acids and nucleoproteins and energy metabolism or calorimetry are exhaustively and rationally dealt with. The connected formulae and reactions find adequate coverage. The medical student will profit by reading through the comprehensive account on proteins, their synthesis, their identification and their clinical significance. The student will greatly benefit by the descriptive account of enzymology and vitamins. The role of inorganic elements inclusive of electrolytes, chemistry of blood, chemistry of respiration, descriptive account and critical evaluation of renal and liver function tests are adequately covered. Succinct account of hormones is given. Valuable information is included on cell, nucleus, membrances and tissues.

This book will be welcomed by the teacher in biochemistry, the medical student at all levels and the specialist in different branches of medicine as well as the general practitioner for revising and refreshing his or her knowledge in biochemistry.

I am confident that this book will go through many editions in the coming years and the author will add more details on topics such as cerebrospinal fluid, lymph, etc.

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PREFACE

The first edition of this book was published in 1974. My aim was to present the subject in a simple, narrative form, covering it comprehensively enough to meet the requirements of the medical students. The book has now gone through four editions and two reprints. This is the fifth edition. Due to the tremendous strides made in the advancement of the subject by intense research the world over, the size of the book necessarily increased from edition to edition to incorporate some of the relevant aspects of the newer knowledge gained. However, the main objective of the book remains the same – a simple presentation of the subject in a comprehensive manner. Every chapter has been thoroughly revised, shedding some obsolete matter and rearranging some of the chapters.

I am thankful to Prof. N. Appala Raju, D. Sc.. Professor and Head of the Department of Chemistry, Sri Venkateswara University, Tirupati and Prof. M.P. Sastry, Ph.D., Professor and Head of the Department of Statistics of the same University, for their help and guidance in writing the chapters on "Some Aspects of Physical and Organic Chemistry" and "Biostatistics".

It is hoped that this edition will satisfy all the requirements of medical students at the undergraduate and post-graduate levels and also the requirements of students of Veterinary Medicine, Agricultural Sciences, Home Science and others who have to take a basic course in Biochemistry.

I am grateful to Dr. D. Jaganatha Reddy, then Vice-Chancellor, Sri Venkateswara University, Tirupati, for his gracious Foreword to the First Edition, and his continuing interest in the subsequent editions.

Last and most important, I thank the successive generations of students whose appreciative response to my teaching and my book gave me the courage to write the book in the first instance and to continue the venture into further editions. I do hope my teacher colleagues find the book useful to their student requirements.

All constructive criticism and comments on the book from students and teachers are most welcome and shall form the basis for preparing future editions.

Tanuku.
June, 1, 1986

A.V.S.S. Rama Rao

INTRODUCTION

SCOPE OF BIOCHEMISTRY

BIOCHEMISTRY is the chemistry of the living matter in its different phases of activity. It probes into the chemical changes involved in the origin of the living matter, follows the changes involved in its growth and studies the processes not only until death but thereafter until dissolution of the matter following its death. The subject encompasses a study of the chemical nature of all living matter from the smallest virus and microorganism to the most complex and highly evolved human being. The relationship of the living to their environment; the processes by which an exchange of chemical substances takes place between the living organism and its environment through digestion, absorption and excretion; the processes by which the absorbed materials are utilized for synthetic reactions leading to growth and replenishment of tissues and multiplication of the cell and the species; the metabolic breakdown of the materials to supply energy for all the above; the mechanisms which regulate with precision all these processes by means of hormonal and neuro-regulatory stimuli — all these come under the purview of biochemistry.

The chemical substances involved are both inorganic and organic and the processes in their entry into and removal from the organism invoke the principles familiar to physical chemistry. Thus the study of biochemistry requires a proper basic knowledge of inorganic, organic and physical chemistry since it has its roots in all the three.

HISTORY OF THE DEVELOPMENT OF BIOCHEMISTRY

Biochemistry as a science is rather young. Any attempts to trace its history must go back to the beginnings of organic chemistry from which it mainly took root.

Theophrastus Bombastus von Hohenheim (1493-1541) — better known as Paracelsus–was the earliest to refer to some chemical aspects of medicine in his writings. Early chemists were mostly seized with the task of finding out the chemistry of plant and animal materials. The Swedish pharmacist Scheele (1742-1786) isolated and studied a number of substances like citric acid, lactic acid and uric acid from biological materials.

Lavoisier (1743-1794) was the earliest to notice that the "burning" inside the body is similar to the combustion and oxidation of organic materials; he realized that oxygen was consumed and carbon dioxide was given out in both instances. This may be said to be the beginning of the modern 'animal calorimetry'. Leibig (1803-1873) showed that plant material depended for its nutrition on simple inorganic substances.

The theory of 'vitalism'–that animal materials fundamentally differed from lifeless substances in that they can be synthesized only by living matter–proved to be only a myth

when Wohler (1800-1882) synthesized urea by simple chemical processes in the laboratory. The earliest book relating to biochemistry was **Lectures in Animal Chemistry** published by the Swedish chemist Berzelius in 1806.

During the later part of the nineteenth century Chevreul, Kossel and Emil Fischer contributed a great deal to the elucidation of the chemistry of fats, proteins and mucoproteins and carbohydrates, the principal constituents of protoplasm. The foundations for enzyme chemistry were laid by the studies on yeast fermentation by Pasteur (1822-1895). Buchner ably pursued these concepts further into the early part of the present century. It is to Michaelis we owe the present theory of enzyme–substrate interactions.

The concepts developed by Arrhenius, Van't Hoff and Oswald about osmotic pressure and electrolytes and the concept of pH developed by Sorenson enabled better understanding of the functioning and homeostasis of the body fluids. Several pioneers have contributed in bringing the subject to its present stage. They used relatively simple tools and analytical methods for their studies and succeeded in unravelling many of the fundamental processes of biochemistry. Meyerhoff and Hill discovered the interrelationship between muscle contraction and lactic acid production; Warburg and others extensively studied the mechanisms of cellular oxidation and helped in the understanding of hydrogen and electron transferring systems in biological oxidations: Lippman recognized the high energy stored in the terminal pyrophosphate linkages of adenosine triphosphate (A.T.P.): Szent–Gyorgi and Krebs elucidated the aerobic oxidation reactions of citric acid cycle.

While these fundamental discoveries were being made, advances in other sciences, notably physics (including optics) and physical chemistry lead to the development of highly sophisticated tools of study and analysis. The electron microscope, the ultracentrifuge, chromatographic and electrophoretic methods of analysis, labelling with radioactive and mass isotopes, X-ray diffraction and a host of others have all contributed to the rapid advance of the subject to its present stage during the last few decades. The scientific and enquiring outlook have ousted superstition and blind belief and research has become an honourable career and got its due recognition from the State and the public paving the way to research by thousands of enthusiastic workers and rapid unravelling of many a hitherto difficult problem. The elucidation of the structure of insulin by Sanger, synthesis of the posterior pituitary hormones by du Vigneaud, elucidation of the structure and enzymic synthesis of nucleic acids by Watson and Crick, Kornberg, Nirenberg and Khorana and the unravelling of the structure of the gene by Jacob and Monod are but a few of the more recent achievements of modern biochemistry.

1

SOME ASPECTS OF PHYSICAL AND ORGANIC CHEMISTRY

Atomic structure: An atom consists of positively charged nucleus surrounded by one or more orbital electrons. The nucleus contains protons and in most cases also some neutrons. The nucleus carries a net positive charge due to the protons and is surrounded by planetary electrons which exactly balance the positive charge on the nucleus with an equal amount of negative charge. An electron has a diameter 1/40,000 of an atom. A proton is even smaller, 1/1840 of the diameter of an electron. The protons and neutrons together i.e. the nucleus make up most of the weight of the atom. Yet the radius of the nucleus is only about 1/10,000 of the radius of the atom. The number of the positive charges on the atomic nucleus is called the *atomic number.*

Isotopes: If a neutron is removed from the nucleus or added to it, it will alter the atomic weight by one unit. But the positive charge on the nucleus does not alter i.e. the atomic number remains the same. The number of planetary electrons also remains unaltered. Elements which have same atomic number but different atomic weights are called isotopes.

Arrangement of Extra-Nuclear Electrons: The electrons revolve round the nucleus in circular or elliptical orbitals. The orbitals are characterized by quantum numbers (n) as 1,2,3 etc. or by the letters of the alphabet starting from K,L,M etc. The maximum number of electrons in each shell around the nucleus is twice the square of the Principal Quantum Number. Thus the maximum number of electrons in the

K shell will be $2x1^2 = 2$
L shell will be $2x2^2 = 8$
M shell will be $2x3^2 = 18$ and so on.

When the outermost shell contains 8 electrons, it confers stability to that element; eg. Neon, Argon etc. Helium has an atomic number of 2 and atomic weight of 4 (represented Symbolically as $_2He^4$. It has one electron shell (K) with 2 electrons. It is also a very stable (in other words a very inert) gas.

In some elements, the electrons in the outermost shell are not very strongly bound and can give rise to positively charged ions and are classed as metals. Also, an electron may take its place either in the outermost shell or move into the next inner shell and give rise to variable electrovalency; eg. $Mn^{\cdot\cdot}$, $Mn^{\cdot\cdot\cdot}$, $Fe^{\cdot\cdot}$ and $Fe^{\cdot\cdot\cdot}$

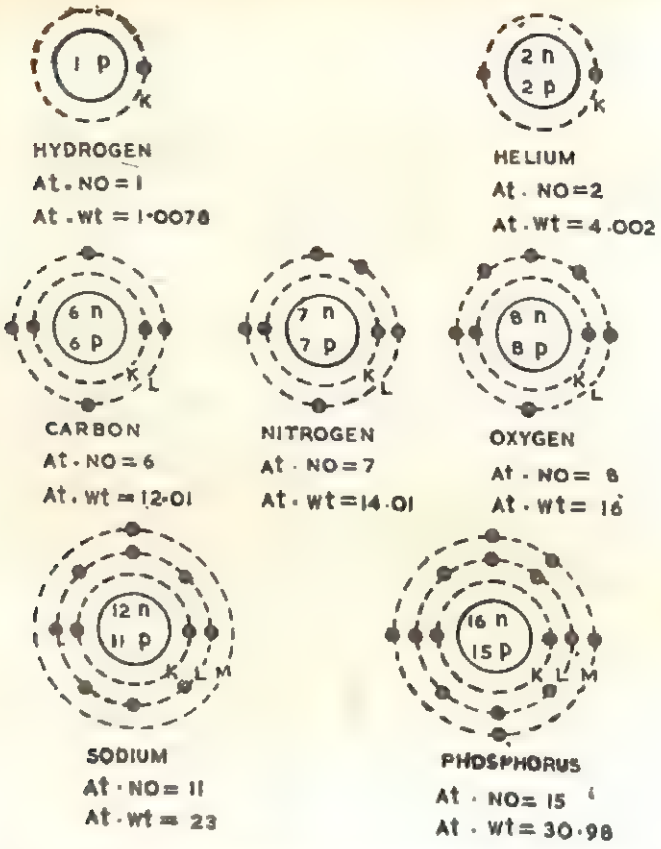


Fig. 1-1. Electronic Configuration of Some Atoms.

The Periodic Law: Just as the year is divided into periods or seasons like summer, rainy season, winter and autumn which repeat themselves from year to year periodically, the elements, as the atomic numbers steadily increase, exhibit physical and chemical properties which show periodic variation. Take, for example, the boiling point of elements: this goes on increasing from hydrogen (atomic no. 1) to reach a maximum for carbon (at. no. 6) and then falls to a low level for nitrogen (at. no. 7) and remains low upto neon (at. no. 10). Then it starts increasing again reaching a peak for silicon (at. no. 14). It comes down again for phosphorus (at. no. 15) and so on. Thus, while the seasons show a periodicity as a function of time, the boiling point of elements shows a periodicity as a function of their atomic numbers. Not only boiling points but many other physical and chemical properties show variations as a periodic function of the atomic numbers of the elements. Accordingly, all elements are divided into seven Periods 1 to 7 (See Fig. 1-2.) Hydrogen, which has unique properties of its own, is placed in period 1. The other periods have Groups of elements varying in number from eight to thirty two. The elements in each Period are divided into eight Groups I to VIII, and a Group O. The number of the Group indicates the number of electrons in the outermost orbital. The transition elements do not exactly fit into this rule. The Group O comprises of the noble gases helium, neon, argon etc. They are not assigned to any of the Periods.

	IA	IIA	IIIA	IVA	VA	VIA	VIIA	Groups VIII			IB	IIB	IIIB	IVB	VB	VIB	VIIB	O
Period 1	1 H																	2 He
Period 2	3 Li	4 Be											5 B	6 C	7 N	8 O	9 F	10 Ne
Period 3	11 Na	12 Mg											13 Al	14 Si	15 P	16 S	17 Cl	18 Ar
Period 4	19 K	20 Ca	21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr
Period 5	37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 (Tc)	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe
Period 6	55 Ca	56 Ba	57-71 Lantha-nides	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn
Period 7	87 Fr	88 Ra	89 Acti-nides															

Lanthanide Series	57 La	58 Ce	59 Pr	60 Nd	61 (Pm)	62 Sm	63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Er	69 Tm	70 Yb	71 Lu
Actinide Series	89 Ac	90 Th	91 Pa	92 U	93 (Np)	94 (Pu)	95 (Am)	96 (Cm)	97 (Bk)	98 (Cf)	99 (Es)	100 (Fm)	101 (Md)	102 (No)	

Fig. 1-2

Avogadro's Number: The weight in grams of 6.02×10^{23} number of particles (atoms, molecules etc.) is the formula weight (atomic weight, molecular weight, etc.) of that substance. Thus 12 grams of carbon (its atomic weight) contain 6.02×10^{23} atoms of carbon. The gram formula weight or molecular weight is commonly referred to as MOLE. A millimole is a thousandth of a mole.

Radioactivity: Certain atomic nuclei are less stable than others. In the process of achieving stability, they emit radiations and are said to be radioactive. The radiation is due to disintegration of their nuclei throwing off a stream of electrons or of helium nuclei. Any loss of protons by an atomic nucleus is termed 'radioactive decay'. The atomic number changes and it becomes a different element altogether. The process is called 'transmutation'. Three types of radiation are described:

Alfa rays: They travel at 1/10 of the velocity of light and each particle consists of two protons and two neutrons (i.e. the nucleus of a helium atom). They cannot travel beyond a few centimetres in air. They are stopped by collision with air particles. They cannot pass through even a cardboard. Uranium is a common source of alfa rays. On the loss of an alfa particle, the uranium (atomic weight 238, atomic number 92) is converted to thorium (atomic weight 234, atomic number 90). The equation for the change is —

$$^{238}_{92}U \longrightarrow {}^{234}_{90}Th + {}^{4}_{2}He$$

The process continues until the stable element, lead, is formed $^{206}_{82}Pb$.

Beta Rays: They are streams of electrons arising from within the nuclei and thrown out of them. They are 1/7,000 in size compared to alfa particles and can penetrate the outer layers of skin. But they cannot reach the internal organs. As a result of loss of an electron from the nucleus, one of the neutrons of the nucleus is converted to a proton. Thorium can emit beta rays as well as gamma rays.

$$^{234}_{90}Th \longrightarrow {}^{234}_{91}Pa + {}^{0}_{-1}\beta + \gamma$$

Gamma Rays: These are not particles. They are a form of energy similar to X-rays. (They usually accompany the emission of alfa rays and beta rays). They are highly penetrating.

Cosmic Rays: They are streams of particles which flow into earth's atmosphere from the Sun and outer space. Their intensity increases during Solar flares. The primary cosmic rays are high speed protons, alfa particles, electrons and larger nuclei. On reaching the earth's atmosphere, they collide with oxygen and nitrogen and produce secondary cosmic rays. The heavier nuclei are broken down and electrons, positrons, protons, neutrons and mesons reach the ground level.

Half-Life: Half-life or $t_{1/2}$ is the time taken for an initial quantity of radioactive material to decay to one half of that amount. For radon, it is about 4 days. For radium, it is about 1,600 years.

Curie (Ci): It is the unit to measure radioactivity. One curie is 3.7×10^{10} disintegrations per second. A millicurie is one thousandth of a curie. A microcurie is one thousandth of a millicurie.

A Becquerel (Bq) = $1/3.7 \times 10^{10}$ of a curie.

Exposure to X-rays and gamma rays is measured in units known as Roentgen (R). It is defined as that amount of X-rays or gamma rays which will produce ions bearing an aggregate electric charge of 2.1×10^{10} units in 1 c.c. of dry air at S.T.P. If a population is exposed to 650 R, it can cause the death of half the population in 1 to 4 weeks and cause permanent and irreversible damage to tissues in the remaining half of the population.

Detection and Measurement of Radiation:

1. *Scintillation Counter:* When hit by radiations, certain substances like zinc sulfide phosphor will give off a tiny flash of light. The flashes can be magnified and counted by suitable devices.

2. *Geiger Counter:* A gas-filled tube called Geiger-Muller tube is used. When a radiation passes through the gas, it ionizes the gas and makes it an electrical conductor. A pulse of ions are generated in the chamber and are conducted through a wire to a detector. The impulses can be counted.

Artificial Radioactivity: When alfa particles from a natural source (say Uranium) stream through nitrogen, a stream of high speed protons are produced due to penetration of the nuclei of nitrogen atoms by the alfa particles and dislodgement of the protons from them. A isotope of oxygen is formed.

$$^{14}_{7}N + {}^{4}_{2}He \rightarrow {}^{17}_{8}O + {}^{1}_{1}H$$

oxygen isotope — proton

Several artificial isotopes like $^{17}_{8}O$ can be produced by bombarding the nuclei of natural elements with particles, particularly high speed neutrons. Many of these isotopes produced by such transmutation reactions have practical applications in diagnostic and therapeutic medicine as well as in research.

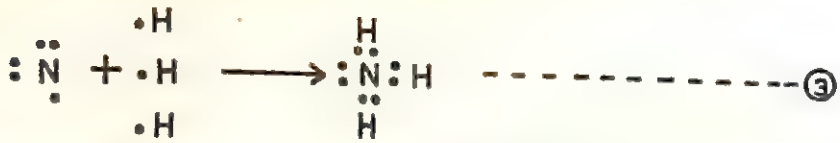
Electro Valency: In chemical combination of elements, it is the outermost shell of electrons which participate to form a stable system. The number of electrons which an atom must gain or lose in order to form a stable system with 8 electrons in the outer shell *(octet)*, or to assume the structure of the nearest inert gas (eg. Helium has only two electrons in the outer shell) is called the *Valence* of that element. Eg. Sodium loses its only electron from its outermost orbital (in this case the third or the M orbital) in forming a compound. The second orbital (L) has 8 electrons (a stable configuration). Hence the valency of sodium is said to be 1. (see Tab. 1-1) Chlorine, which has 7 electrons in the outermost orbital (M) can take up one electron to make it an octet. Its valency is also one. Na and Cl can thus readily combine by transfer of an electron from the outer shell of sodium to the outer shell of the chlorine atom. Sodium is said to be electropositive (since it loses an electron, a negatively charged particle) and chlorine is electronegative (since it gains a negative charge – an electron). Such compounds formed by transfer of electrons between the participating elements are called *Electrovalent Compounds* or *Polar Compounds.*

Covalency: Here the electrons are not transferred from atom to atom, but shared between two atoms. Thus, hydrogen atom, which has only one planetary electron, comes together with another hydrogen atom and the two hydrogen atoms share the two electrons between themselves, to form a *duplet.* This is a stable structure and is called the *hydrogen molecule.*

$$H. + H. \longrightarrow H : H \text{ ---------- } 1$$

Similarly, chlorine with seven outer orbital electrons can come together with another chlorine atom and by sharing one electron between themselves can form an octet and a stable molecule of chlorine.

Nitrogen, which has 5 electrons in the outer shell, combines with three atoms of hydrogen and shares their electrons to form an octet, the resultant compound being NH_3.



In this combination, each hydrogen atom has a duplet (the stable configuration of Helium) and the nitrogen atom has an octet.

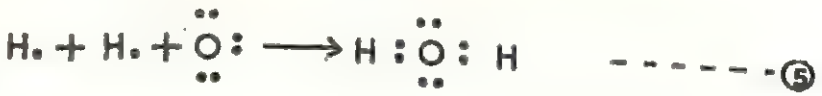
Similarly, in the molecule of oxygen, two pairs of electrons are shared by two oxygen atoms.

When two electron pairs are shared, it is represented as a double bond. If three pairs are shared, it is represented as a triple bond.

All these are examples of *Covalent Bonds.*

Co-ordinate or Dative Valency: In some cases, the two shared electrons forming a linkage may be more attracted to one of the two participating atoms. It is then called a *co-ordinate* or *dative valency*. The atom with less affinity for the electrons (the donor atom) shows a net positive charge, while that with greater affinity for the electrons (the acceptor atom) carries a negative charge. The molecule becomes *polar* and is called a *"Dipole"*.

In a molecule of water, oxygen atom has greater affinity for the electrons and this is shown by placing the duplets nearer to 'O' than 'H'.



A few molecules of water (one out of several million) may actually ionize as H^+ and OH^-, the covalent bond becoming an electrovalent bond.

When polarized molecules are placed in an electric field between a positive pole and a negative pole, their molecules arrange themselves in a head-to-tail series and decrease the strength of electric attraction between the two poles. The capacity of a substance to decrease the strength of electric attraction between two poles is called the *"Dielectric Constant"* of that substance.

Ionic compounds like NaCl ionize readily in a solvent of high dielectric constant (like water) and do not ionize in solvents of low dielectric constant (eg. benzene). Because of high dielectric constant, water molecules decrease the attraction between Na^+ and Cl and keep them apart in an ionized state.

The structure of some of the biologically important elements is shown in Table—1-1,

TABLE 1-1

Element	Symbol	*Atomic weight	Atomic number	Planetary Electrons in K,L.M, etc. shells
Hydrogen	H	1.008	1	1
Helium	He	4.003	2	2
Carbon	C	12.011	6	2-4
Nitrogen	N	14.008	7	2-5
Oxygen	O	16.000	8	2-6
Sodium	Na	22.991	11	2-8-1
Magnesium	Mg	24.32	12	2-8-2
Phosphorus	P	30.975	15	2-8-5
Sulfur	S	32.066	16	2-8-6
Chlorine	Cl	35.457	17	2-8-7
Potassium	K	39.100	19	2-8-8-1
Calcium	Ca	40.08	20	2-8-8-2
Iron	Fe	55.85	26	2-8-14-2
Cobalt	Co	58.94	27	2-8-15-2
Copper	Cu	63.54	29	2-8-18-1
Zinc	Zn	65.38	30	2-8-18-2
Iodine	I	126.91	53	2-8-18-18-7

* The atomic weights are not round numbers, because small amounts of isotopes are always present with slightly lesser or greater atomic weight than the predominant isotope. The atomic weight is hence an average of the aggregate.

TABLE 1-2

Elementary composition of the human body:

Element		*Per cent*
H	...	*63.00*
O	...	*25.50*
C	...	*9.50*
N	...	*1.40*
Ca	...	*0.31*
P	...	*0.22*
Cl	...	*0.08*
K	...	*0.06*
S	...	*0.05*
Na	...	*0.03*
Mg	...	*0.01*

The most abundant elements in the living organisms are hydrogen, oxygen, carbon and nitrogen. They make up 99% of the mass of most cells. (see table 1-2). These elements have unique properties.

1. They readily form covalent bonds by electron sharing. Hydrogen needs one electron, oxygen two, nitrogen three and carbon four to complete their outer electron shells and thus form stable covalent bonds. They are the lightest elements capable of forming covalent bonds and the bonds so formed are quite strong. Carbon, nitrogen and oxygen can share either one or two electron pairs to yield either single or double bonds.

2. Simple, low molecular weight molecules like CO_2, N_2 and H_2O are taken up by the living organisms from the atmosphere and are synthesized into the simple building blocks like glucose, nucleotides, amino acids and fatty acids. From these, the large macromolecules like polysaccharides, nucleic acids, proteins and lipids are formed. They may further combine to form *supramolecular systems* such as lipoproteins and ribosomes. These components of supramolecular systems are held together, not by covalent bonds, but by relatively weak, non-covalent forces like hydrogen bonds, hydrophobic interactions and van der Walls interactions. Though the individual bonds are weak, on account of their very large numbers, they give sufficient stability to the supramolecular complexes.

3. The supramolecular systems are further assembled together into cell organelles like nuclei, mitochondria, chloroplasts and others. Here, again, the holding force is mainly by noncovalent interactions.

Nucleic acids and proteins are called the 'Informational Macromolecules'. Each nucleic acid and each protein has got a specific nucleotide or amino acid sequence which contains the information about its functional role in the cell. On the other hand, polysaccharides and lipids have a relatively simpler structure and do not have any function of carrying information.

SOME IMPORTANT PROPERTIES OF WATER

90% of blood plassma is water and 60-80% of tissues is also water. Water has very distinctive properties compared to other liquids. It has a high boiling point, high specific heat of vaporization and a high melting point. This is on account of strong intermolecular forces acting between adjacent molecules of H_2O. It is in a highly polarized state due to the greater attraction of the electrons to oxygen which hence becomes electronegative, leaving the hydrogen electropositive. This results in each water molecule being surrounded by four other water molecules, with the negatively charged oxygen atoms being attached to the neighbouring positively charged protons, as shown in Figure 1-3. The oxygen atom forms a tetrahedron with four hydrogen atoms, two of its own and one each from two neighbouring water molecules. The H-O bond is known as the hydrogen bond.

Solvent Properties of Water: Water disperses or solubilizes into the form of miscelles many compounds which contain both strongly polar and nonpolar groups. Such molecules

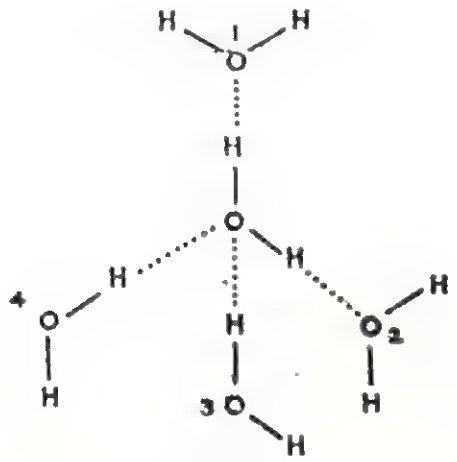


Fig. 1-3

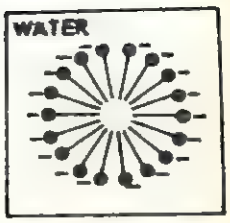


Fig. 1-4

are called amphipathic. eg sodium oleate or stearate. The carboxyl group is polar while the long hydrocarbon tail is nonpolar. When sodium salt of the fatty acid (soap) is added to water, it does not form a true ionic solution, but disperses in water to form miscelles in which the negatively charged carboxylate groups are exposed and form hydrogen bonds with water molecules. The nonpolar hydrocarbon chains are hidden within the miscelle. The soap miscelles have a net negative charge and remain suspended because of mutual electrostatic repulsion. Within the miscelle, adjacent hydrocarbon structures are held together by van der Walls interactions. (see Fig. 1-4)

There is no true stoichiometric bonding between the hydrocarbon tails in the miscelles. Hence it is a hydrophobic interaction and not a hydrophobic bond.

The amount of heat energy (expressed as calories) required to raise the temperature of one gram of water at 15° to 16° is known as the *specific heat* of water. It is one calorie and is quite high compared to other solvents. (eg. Ethanol, 0.58 calorie; acetone, 0.53 calorie; chloroform, 0.23 calorie). It is therefore best suited to maintain constant temperature of the body with varying environmental temperatures. The high heat of vaporization (540 cal/gram at 100° compared to say 204 cal/gram for alcohol and 59 cal/gram for chloroform) is also helpful in maintaining body temperature. By vaporization of only a small amount of water from the surface of the skin (in the form of sweat), a large amount of body heat can be dissipated.

Water has maximum density at 4°, whereas it solidifies and forms ice only at O°. Ice is hence lighter than water at 4° and floats on the surface, thus allowing the aquatic life to continue below the ice. It also facilitates easier melting of the ice exposed to the atmosphere when the environmental temperature rises.

Hydrogen Ion Concentration: Water can be considered to be a weak electrolyte. It can dissociate as follows:

$$H_2O \longleftrightarrow H^+ + OH^-$$

The equilibrium constant for the above dissociation is given by the equation

$$K_{eq} = \frac{(H^+)(OH^-)}{(H_2O)}$$

At 25°, it is found to be 1.8×10^{-16}

In pure water, the molecular concentration of water is $\frac{1000}{18}$ i.e. 55.5 moles/litre.

$$(H^+) \times (OH^-) = 55.5 \times 1.8 \times 10^{-16} = 1.01 \times 10^{-14}$$

The product $(H^+) \times (OH^-)$ is called the *ionic product* of water, abbreviated as K_w.

$\therefore K_w = 1.01 \times 10^{-14}$ at 25°.

In pure water, equal number of hydrogen and hydroxyl ions exist, i.e. $(H^+) = (OH^-)$.

$\therefore (H^+) \times (OH^-) = (H^+) \times (H^+) = (H^+)^2 = 1.01 \times 10^{-14}$
$\therefore (H^+) = 1.01 \times 10^{-7}$

pH: For ease of representation of (H^+) in numbers rather than as fractions, Sorenson developed the concept of pH. pH is defined as.the logarithm of the reciprocal of hydrogen ion concentration.

$$pH = \log \frac{1}{(H^+)} = -\log (H^+).$$

For pure water, since $(H^+) = 1.0 \times 10^{-7}$, pH will be 7.0.

Acids and Bases: An acid is a proton donor and a base is a proton acceptor (Bronsted—Lowry).

$$CH_3COOH \longleftrightarrow CH_3COO^- + H^+$$

Acetic acid is hence a proton donor and an acid. Acetate (CH_3COO^-) is a proton acceptor and hence a base. The two together constitute a conjugate acid—base pair. In dilute aqueous solutions an acid will dissociate to give a proton which is taken up by a water molecule to from a hydronium ion, H_3O^+

$$HA + H_2O \longrightarrow H_3O^+ + A$$

Acids which have only a slight tendency to give up protons are weak acids (eg. acetic acid). Acids which give up their protons readily to water are strong acids (eg.hydrochloric acid).

DISSOCIATION OF SOLUTIONS IN WATER

Dissociation of strong electrolytes: Strong acids, bases and their salts are called strong electrolytes. They dissociate almost completely in water.

$$HCl \longrightarrow H^+ + Cl^-$$
$$NaOH \longrightarrow Na^+ + OH^-$$
$$NaCl \longrightarrow Na^+ + Cl^-$$

Dissociation of weak electrolytes: Weak acids, weak bases and their salts are called weak electrolytes. They dissociate only slightly in solution.

Taking acetic acid as an example-

$$CH_3COOH \longrightarrow CH_3COO^- + H^+$$

The equilibrium constant for this acid is 1.8×10^{-5}

i.e. $$\frac{(H^+)(CH_3COO^-)}{(CH_3COOH)} = 1.8 \times 10^{-5}$$

The pH of a 1.0 molar solution of acetic acid can be calculated from the above equation to be 2.38.

Henderson-Hasselbalch Equation:

$$HA \longleftrightarrow H^+ + A^-$$

The Equilibrium Constant K_{ion} or $K_a = \frac{(H^+)\ (A^-)}{(HA)}$

$$\therefore (H^+) = K_a \frac{(HA)}{(A^-)}$$

$$\therefore \text{Log}\ (H^+) = \text{Log}\ K_a + \text{Log}\frac{(HA)}{(A^-)}$$

Multiplying both sides by (— 1), we have

$$-\log(H^+) = -\text{Log}\ K_a - \text{Log}\frac{(HA)}{(A^-)}$$

But — log (H^+) =pH; — log Ka is defined as pKa;

and $$-\log\frac{(HA)}{(A^-)} = \log\frac{(A^-)}{(HA)}$$

Substituting in the above equation,

$$pH = pKa + \log\frac{(A^-)}{(HA)}$$

pKa is the dissociation constant of the weak acid HA, (A^-) and (HA) are the molar concentrations of the salt of the weak acid and the weak acid respectively.

Titration Curves: A weak acid, say 100 ml of 0.1 N CH_3COOH, can be titrated with a strong base, say 0.1 N NaOH, and the continously varying pH can be plotted on the Y– axis against the ml of 0.1 NaOH added from time to time on the X-axis. A curve as shown in Figure 1–5 is obtained. It may be noted that the rate of change of pH per unit addition

of alkali is highest at the beginning and towards the end of titration. The smallest rate of change occurs at the midpoint of the titration. At this point, there will be equimolar amounts of acid and its salt formed with the base (say acetic acid and sodium acetate). There is minimum change of pH when an acid or base is added to such a mixture. The ability of a solution to resist a change in pH is referred to as its "buffering action". The pH at the midpoint of titration represents the *dissociation constant* of the acid.

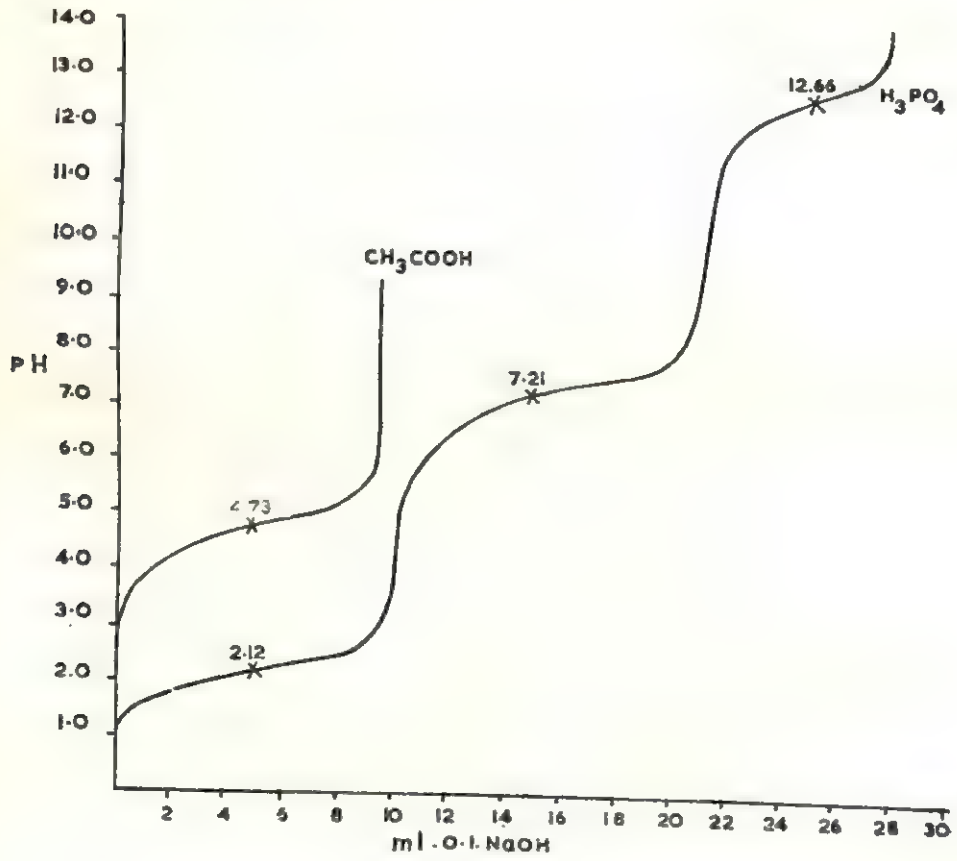


Fig 1-5

$$\text{In the equation pH} = \text{pKa} + \log \frac{(A^-)}{(HA)}, \quad (A^-) = (HA)$$

$$\log \frac{(A^-)}{(HA)} = \log \frac{1}{1} = 0$$

$\therefore$ pH=pKa, when $(A^-)=(HA)$.

The pKa or the dissociation constant of an acid varies from acid to acid and is a characteristic property of each acid.

If, instead of a mono–basic acid like CH_3COOH, a poly-basic acid is titrated, we get a different type of titration curve. If phosphoric acid is titrated,

$H_3PO_4 \longleftrightarrow H^+ + H_2PO_4^-$ Kal=7.5×10^{-3}, pKa=2.12

$H_2PO_4^- \longleftrightarrow H^+ + HPO_4^=$ Ka2=6.23×10^{-8}, pKa=7.21

$HPO_4^= \longleftrightarrow H^+ + PO_4^{\equiv}$ Ka3=2.2×10^{-13}, pKa=12.66

At the respective pKa values, the first, second and third ionizations are half complete. The titration curve for phosphoric acid can be seen in Figure 1-5.

Ionization of Bases: The ionization of a base is similar to that of an acid.

$$BOH \longleftrightarrow B^+ + OH^-$$

The equilibrium constant for this reaction Kb and the dissociation constant pKb have similar meanings as for acids. Like pH for acids, pOH can be calculated for bases.

$$POH = pKb + \log \frac{(B^+)}{(BOH)}$$

The above equation can be solved as in case of pH.

$$pOH = -\log (OH^-)$$

Buffers: A buffer solution is one which resists a change in pH when an acid or base is added to it. It is usually made up of a mixture of a weak acid and its conjugate base (salt of the weak acid) eg. acetic acid and sodium acetate. To such a mixture, suppose an alkali is added. It will react with the weak acid to form its salt.

$$NaOH + CH_3COOH \longrightarrow CH_3COONA + H_2O$$

On the other hand, if an acid is added, this will be taken up by the base sodium acetate.

$$HCl + CH_3COONa \longrightarrow CH_3COOH + NaCl$$

In either case, there is no increase in either H^+ or OH^-. The relative concentrations of the acetate and acetic acid are altered slightly. But, as seen from the titration curves in Fig. 1-5 there is little change in the pH on this account, since the buffer represents the mid-zone of the titration curve where change is minimal for addition of acid or alkali.

The *capacity* or efficiency of a buffer depends on the molar concentrations of the buffer components. O.1 M acetate buffer means a solution containing acetate and acetic acid whose concentrations together make O.1 M per litre e.g. 0.05 M sodium acetate and 0.05 M acetic acid, or 0.04 M sodium acetate and 0.06 M acetic acid. The effectiveness is most when the concentrations of acid and base are equal. At this point, the pH of the buffer will be the same as the pKa of the acid.

Colligative Properties of Solutions: The properties which depend only on the concentrations of the solute or solvent and do not depend on their chemical nature are defined as their colligative properties.

1. *Lowering of the Vapour Pressure:* A non-volatile solute lowers the vapour pressure of the solvent, (because it interferes with the escape of the solvent molecules.)

2. *Elevation of the Boiling Point:* A solution has a higher boiling point than the pure solvent. The elevation of the boiling point varies directly as the number of particles

dissolved in it. Hence ionizing substances like NaCl have a greater effect than non-ionizing substances like sucrose. If I mole of NaCl in 1 litre of water raises the boiling point by 1°C, 1 mole of sucrose in 1 litre of water will raise it only by 0.5°C.

3. *Lowering of Freezing Point:* For every mole of a solute dissolved in 1 litre of water, the freezing point is lowered by 1.9°C. As in the case of boiling points, ionizing substances exert a greater effect. Thus a molar solution of $CaCl_2$ has a freezing point 3×1.9 i.e. 5.7°C lower than that of pure water.

4. *Osmotic Pressure:* If two solutions of different particle concentration are separated by a semipermeable membrane, a flow of the solvent particles will occur from the side of the lower particle concentration to that of the higher. The movement will continue till the concentration of the solute on the two sides is the same. The process is known as *osmosis*. If external pressure is applied on the side of higher solute concentration, osmisis can be prevented. The pressure that has to be applied to prevent osmosis is called the osmotic pressure. Osmotic pressure is proportional to the molar concentration of the solute.

Dialysis: If the porosity of the semipermeable membrane is such that large colloidal particles like proteins cannot pass through the pores but smaller organic and inorganic molecules can pass through (eg. cellophane) the colloid particles will exert an osmotic pressure called the 'colloid osmotic pressure'. If a mixture of colloidal particles like protein and crystalloid particles like NaCl is kept in a bag made up of a semipermeable membrane and is suspended in water, the NaCl molecules will pass into the water leaving the protein in the bag. This process of separation of colloids from crystalloids using a semipermeable membrane is known as 'dialysis'.

Kinetic Theory: All gases exert pressure, which is the force exerted by it per unit area. The atmosphere, which is a mixture of gases (mainly oxygen, nitrogen, water vapour and carbondioxide) exerts a pressure equivalent to 760 mm of mercury at O°c and sea level.

Boyle's Law: At a constant temperature, the volume of a given weight of a gas varies inversely as the pressure.

$V \propto 1/P$ or $PV=C$ (V=volume of the gas P=pressure exerted by the gas and C=constant).

Charles' Law: At constant pressure, the volume of a given weight of a gas is direcly proportional to its absolute (Kelvin) temperature.

$V \propto T$ or $V/T=C$ (T=absolute (Kelvin) temperature i.e. 273+temperature in centigrade). Combining the above two laws, we get the equation, for a given weight of a gas -

$$PV/T=C$$

This equation is used to calculate the volume of any given gas under standard (or normal) conditions of temperature and pressure (S.T.P. or N.T.P.) which are defined as 273° absolute temperature (i.e. 0° centigrade) and 760 mm mercury pressure.

Dalton's Law of Partial Pressures: In a mixture of two or more gases which do not react chemically, each gas exerts a pressure which it would have exerted had it been the only gas occupying the entire volume of the mixture. Alternately, in a mixture of two or more gases, the total pressure exerted by the mixture is the sum total of the individual partial pressures.

$$P_t = P_1 + P_2 + P_3 \ldots \text{etc.}$$

where P_t is the total pressure and P_1, P_2 etc., are individual pressures of the gases in the mixture.

The volume occupied by one mole of any gas under S.T.P. is constant and is 22.4 litres.

SOME ASPECTS OF ORGANIC CHEMISTRY

Organic chemistry is the chemistry of carbon compounds. Carbon is a tetravalent element. Its four valencies are directed to the four corners of an imaginary, regular, tetrahedron, the carbon atom itself occupying the centre of such tetrahedron. The structure of methane (CH_4) is shown in Fig. 1-6 as an example. The angle between any pair of bonds is 109.5° and the length of each bond is 0.109 nm.

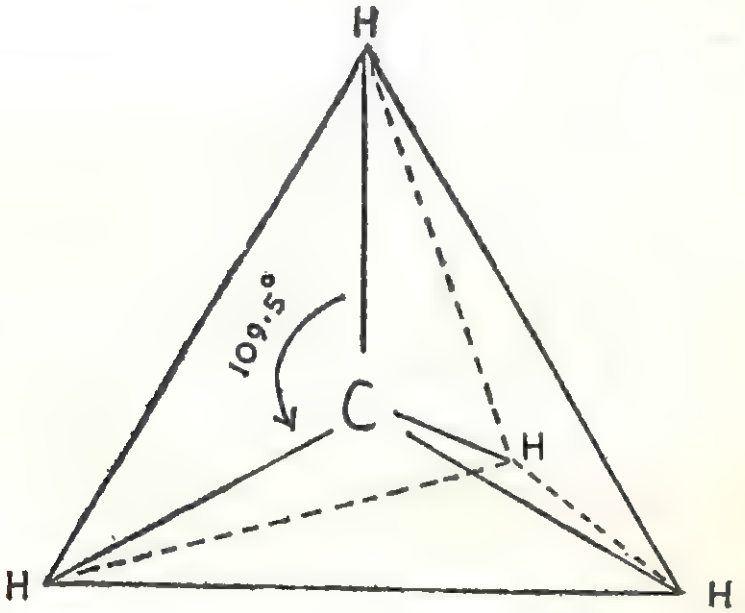
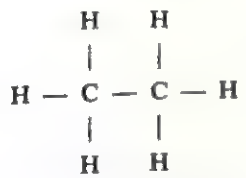


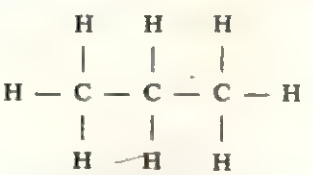
Fig. 1-6

One of the valency bonds may unite with a neighbouring carbon to form a chain of carbons *(aliphatic compounds)*.

eg.



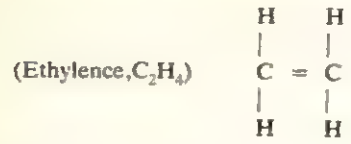
(Ethane, C_2H_6)



(Propane, C_2H_2)

Carbon atoms may also be linked by two or three valencies to form double or triple bonds.

(Ethylence, C_2H_4)



(Acetylene, C_2H_2) $HC \equiv CH$

The carbon atoms can also combine to form a ring structure, as in benzene. These compounds will form the aromatic and heterocyclic organic compounds.

The structure of the benzene ring is shown in Fig. 1-8. The double bonds can alter in position to give either the 'a' type or the 'b' type of ring. This is a phenomenon called "*resonance*". To indicate the variability of the positions of the double bonds, the benzene ring is often represented with a circle in the centre.

Isomers: Compounds which have same elemental composition and same empirical formula, but different molecular structure are called isomers.

eg. $CH_3 - O - CH_3$ (Dimethyl ether)

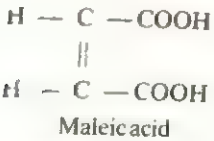
$CH_3 CH_2 OH$ (Ethyl alcohol). Both have the same empirical formula – C_2H_6O.

Isomerism can be of two broad types–structural isomerism and stereo isomerism.

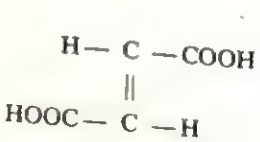
1. Structural Isomerism: The example given earlier of dimethyl ether and ethyl alcohol is one of structural isomerism. Here the component atoms of one compound are combined in a different manner from that of the other. Their molecular formulae will be same, but they differ much in their physical and chemcial properties.

2. Stereoisomerism: Here, not only the molecular formula, but even the groups are same. But the relative positions of the groups in space differ. The properties of the isomers will be similar on account of the same groups, but differ in some other respects due to differences in the spatial arrangements of the groups. Two types of stereoisomerism are described.

a) Geometrical Isomerism:



Maleic acid

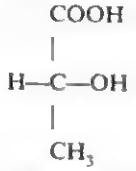


Fumaric acid

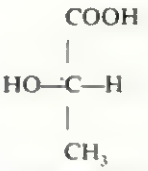
The arrangement of the –H and –COOH groups in maleic acid is on the same side, whereas in fumaric acid, they are arranged on opposite sides. Since the two carbons are linked by a double bond, free rotation between the carbons is not possible. Maleic acid is called the "Cis" isomer and fumaric acid the "Trans" isomer. This is known as "*Cis-Trans*" isomerism.

Similarly, glucose can exist in two different forms–alfa and beta. Here the carbons are held rigidly in a ring form and cannot rotate. (See Fig. 2-1)

b) Optical Isomerism: This is another type of stereo isomerism. A molecule is said to be asymmetric if there is no plane around which there is symmetry in the molecule. This will happen if, in a carbon compound, four different groups are attached in the tetrahedral structure. Lactic acid is a simple example of such asymmetry.



D-Lactic acid



L-Lactic acid

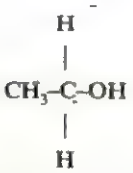
They are mirror images of each other. If a beam of polarized light is passed through their solutions, the D–lactic acid will rotate the plane of polarized light to the right, while the L-lactic acid will turn it to the left. D-Lactic acid is said to be dextrorotatory and L-lactic acid levorotatory. Each compound is said to be the "*Enantiomer*" of the other. A mixture of equal quantities of the D–and L–compounds is called a "*Racemic*" mixture. In such a mixture, no rotation of polarized light can occur as the individual rotations to opposite sides are neutralized.

When there are more than one asymmetric carbons in a molecule, there will be more of optical isomers. In general 2^n gives the number of possible isomers, where 'n' stands for the number of asymmetric carbons in the compound.

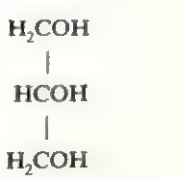
SOME CLASSES OF ORGANIC COMPOUNDS RELEVANT TO BIOCHEMISTRY

Hydrocarbons: They are compounds containing carbon and hydrogen e g. methane, ethylene, acetylene (whose structures are already shown) and their derivatives, like chloroform — $CHCl_3$

Alcohols: They are hydroxy derivatives of hydrocarbons. eg. CH_3OH (methyl alcohol); C_2H_5OH (ethyl alcohol). The –OH group is polar, while the hydrocarbon part is non–polar. The lower alcohols with small number of carbons are freely miscible with water in all proportions. As the number of carbons increases in the higher alcohols, they become altogether insoluble in water and practically non-polar. If the carbon atom bearing the –OH group is attached to only one other alkyl group, it is said to be a primary alcohol. If it is attached to two alkyl groups, it is a secondary alcohol.



Ethyl alcohol
(Primary alcohol)



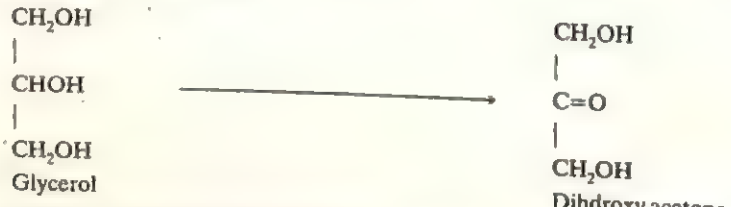
Glycerol
(The alcoholic groups attached to carbons 1 and 3 are primary; the one attached to carbon 2 is secondary).

Substances like glycerol with more than one alconolic group are called polyhydric alcohols.

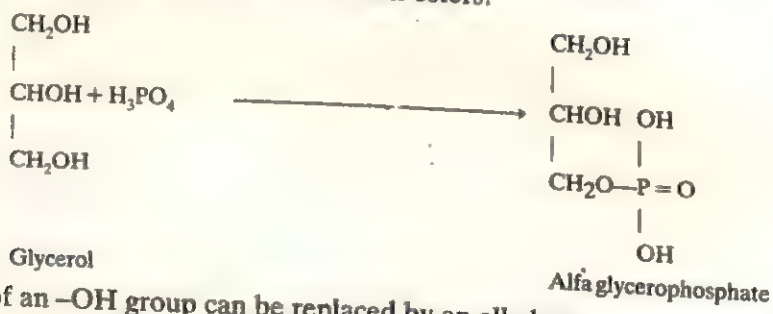
a) Oxidation of alcohols: Primary alcoholic groups, on oxidation, give aldehydes first and then the corresponding carboxylic acids.

$$\underset{\text{Ethyl alcohol}}{CH_3CH_2OH} \longrightarrow \underset{\text{Acetaldehyde}}{CH_3CHO} \longrightarrow \underset{\text{Acetic acid}}{CH_3COOH}$$

Secondary alcohols give ketones.



b) Alcohols can combine with acids to form esters.

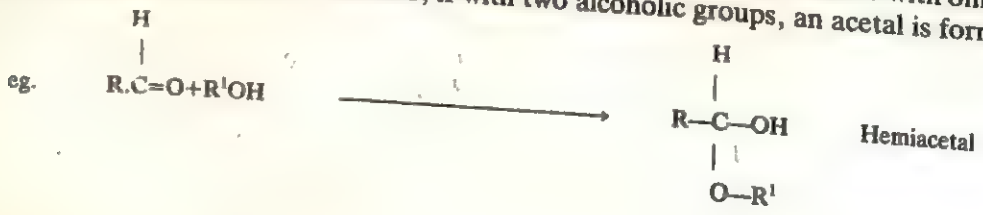


c) The H of an –OH group can be replaced by an alkyl group to form an ether.

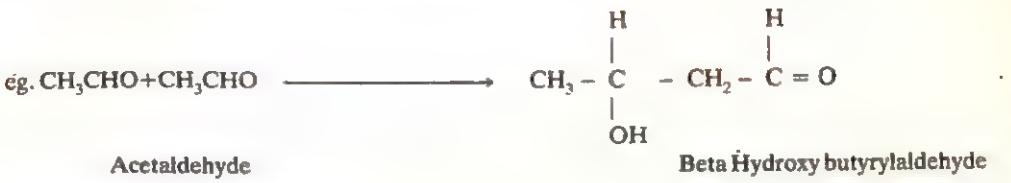
$$\underset{\text{Methyl alcohol}}{CH_3OH} + -CH_3 \longrightarrow \underset{\text{methyl ether}}{CH_3OCH_3}$$

Aldehydes and Ketones: As seen earlier, they can be formed by oxidation of primary and secondary alcohols. Conversely, thay can be reduced to form primary and secondary alcohols, by a reversal of the reaction in (a) above. The aldehydes can be oxidized to form carboxylic acids (see (a) above). But the ketones resist oxidation.

In an acidic medium, aldehydes can combine with alcohols. If they combine with only one alcoholic group, a hemiacetal is formed; if with two alcoholic groups, an acetal is formed.

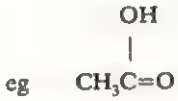


In a basic medium, the aldehyde molecules can undergo condensation between the carbonyl group and the alfa carbon of the alkyl group. This is called *"aldol condensation."*



The ketones are less active in this respect.

Carboxylic Acids: They have a carbonyl group and a hydroxy group attached to the same carbon.



They are weak acids and dissociate only to a small extent in solution.

The negative charge is shared equally between the two oxygen atoms.

Carboxylic acids can be reduced to form corresponding aldehydes [reverse of reaction (a)].

They can form esters with alcohols (see under Alcohols). They can form salts with bases and amides with ammonia.

Two molecules of a carboxylic acid can combine with the elimination of a molecule of water to form an *anhydride*.

SYNTHETIC POLYMERS

Polymers are high molecular weight substances made up of small repeating units (poly means many and meros means parts in Greek). They are macromolecules. The simpler units are called monomers. If the macromolecule is built from a single monomer, it is called a homopolymer. If two are more monomers are present, it is a copolymer. The polymer may be linear in configuration or may be cross-linked and form an interlacing network.

A. Polyolefines: The monomer is an alkene (olefine).

eg. Polyethylene $(CH_2=CH_2)_n$

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It is chemically inert but is permeable to oxygen and carbondioxide. It is used in the manufacture of funnels. tumblers, disposable syringes etc. It is also used for the drainage of wounds.

2. Polypropylene: $-CH_2-\underset{\displaystyle CH_3}{\underset{|}{CH}}-$ is the repeating unit.

It can be used like polyethylene. In addition, it is also used in the manufacture of carpets, heart valve transplants etc.

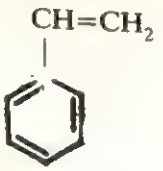
B. Polymers of Dienes: Natural rubber is a polymer of isoprene units.

$$CH_2=\underset{\displaystyle CH_3}{\underset{|}{C}}-CH=CH_2$$

C. Elastomers:

These polymers exibit the property of elasticity. They are olefine copolymers mostly eg. isoprene and isobutylene. Vulcanization is a process by which elastomers like rubber are made tougher and more resistant to abrasion by mixing with sulfur and heating.

D. Polystyrene: It is derived from styrene.



E. Polyvinyl chloride (PVC):

Repeating unit is $CH_2=CHCl$

It is used in the manufacture of hard, transparent plastic bottles, Tygon tubing etc.

F. Teflon: It is a polymer of tetrafluoroethylene.

$F_2C = CF_2$

It is attacked only by molten sodium or potassium and resists all other corrosive substances. It is non-sticking and self-lubricating and has a variety of applications in surgical materials (sutures, drains), burette stopcocks, stoppers (interchangeable) for volumetric flasks etc.

G. Acrylates: Polymers of acrylic acid or derivatives. Repeating unit is $CH_2=CH\ COOH$. It is used in the manufacture of plexiglass, contact lenses, and in plastic surgery.

H. Epoxies: Epoxides, (which are three membered heterocyclic ethers), and their derivatives form the repeating unit.

Epoxy resins are used to cement together a wide variety of materials — wood, glass etc.

1. Polyurethanes: Polymers of diols and diisocyanates. They are used in the manufacture of spongy materials, foam beds etc.

a) Polyamides: They form long fibres. eg. Nylon.
Nylon–66 is a polymer of hexamethylene diamine and adipic acid.

b) Polyesters: Eg. Dacron. The repeating units are ethylene glycol and terepthalic acid. Used in the manufacture of vascular grafts.

Polysiloxanes: Silicones: Alternating silicon and oxygen atoms with alkyl or aryl side chains. They are extremely stable to heat and combustion. They are also water-repellent. They are used to coat materials to make them non-wettable.

SOME ASPECTS OF PHYSICAL CHEMISTRY

Colloidal State and Membrane Phenomena: A colloid system consists of two phases, a continuous dispersion medium and a discontinuous dispersed phase of particles with diameters ranging from 1 to 5 mμ to 100 to 500 mμ. Each particle may be a single molecule or an aggregate of molecules. If a ray of light is passed through a colloidal solution and viewed laterally to the beam of light, it will be seen as an yellow track in the solution. This is due to scattering of light by the colloidal particles, an effect known as 'Tyndall Phenomenon'. When particles of a solid exist in a colloidal system, the surface area of the substance is enormously increased compared to its area when present in the solid state. Depending on the nature of the dispersion medium (solid, liquid or gas) and the dispersion phase (solid, liquid or gas), eight different types of colloidal systems are possible. The biologically important systems are – 1. solid in liquid and 2. liquid in liquid. The solid in liquid systems are also called *suspensoids or lyophobic* colloids. There is little attraction between the dispersion phase and the dispersion medium (hence lyophobic). The liquid in liquid systems are called *emulsoids* or *lyophilic* colloids. There is attraction between the dispersion phase and the dispersion medium. The two liquids are mutually soluble in each other. If water is the dispersion medium, the colloidal particles will be solvated. Colloidal solutions of gelatin in water and proteins in water are examples of emulsoids. In biological matter, water is the dispersion phase. Hence they are called lyophilic or more specifically hydrophilic.

Emulsions: Dispersions of oil droplets in water and vice versa are called emulsions. eg. milk and egg yolk.

Gels: In lyophilic colloidal solutions(sols), when the concentration of the particles exceeds certain limits, a gel is formed. eg. gelatin solution. Coagulation of blood is itself an example of gel formation.

The Electric Charge of Colloidal Particles: The colloid particles are electrically charged. The charges arise from ionization of groups on the particle surface (eg. proteins) or selective adsorption of ions from the medium or both. This charge on the colloidal particles is neutralized by the formation of charges with the opposite sign in the fluid medium

surrounding the particles. The medium will form two layers (Helmholtz–Gouy theory), one immediatley absorbed on the surface of the colloid that is immobile and another that is more diffuse and labile next to the immobile layer. When electrolytes are added, these double layers of the colloidal particles collapse and the colloidal particles aggregate and precipitate. The electrolytes also cause dehydration of the colloid particles by removing the surrounding layer of fluid with which the particle has combined. The process is known as salting out. Addition of alcohol aids precipitation of colloids by removing the water layer surrounding the particles. Small amounts of electrolytes which are ineffective on their own can precipitate a colloid in the presence of alcohol.

Properties of Membranes: The cell membrane represents an interface and, according to Gibb's principle, substances which lower surface tension tend to concentrate there. Thus the membrane contains phospholipids, soaps, cholesterol and other lipids in higher concentrations than in the cytoplasm. The molecules also show a characteristic orientation in the membrane. Substances like phospholipids and proteins ionize on the membrane surfaces and make them electrically charged. There is also a potential difference between the external and internal surfaces of the membranes. This potential difference will persist as long as cellular oxidations are occurring. Under anaerobic conditions, the potential difference disappears. Not only the electric charge, but the selective permeability of the cell membrane depends on the metabolic activity of the cell. The erythrocyte membrane is impermeable to Na^+ and K^+. There is a high concentration of K^+ in the cell and Na^+ in the plasma. But the membrane is freely permeable to Cl^- and HCO_3^- ions. Under conditions of anoxia, however, the K^+ passes out of the cell and Na^+ passes into the cells. On account of the high lipid content of the cell membranes, fat soluble substances pass readily through the cell membranes.

Donnan Membrane Equilibrium: If, on either side of a semipermeable membrane, solutions of different concentrations of sodium choloride are kept, the Na^+ and Cl^- ions from the more concentrated side will pass to the less concentrated side till the concentrations become equal.

$$4\,NaCl \quad | \quad 2\,NaCl \qquad\qquad 3\,NaCl \quad | \quad 3\,NaCl$$

If, on the other hand, we have a non-diffusible sodium salt on one side (NaR) and NaCl on the other side (as in A in Fig. 1-7), Na^+ions can diffuse either way, but Cl^-ions can diffuse only to the left and R^-ions cannot diffuse at all. After some time, when equilibrium is reached, (see B in Fig. 1-7), the product of $Na^+ \times Cl^-$in one compartment will be equal to the product of those two ions in the other compartment.

$$(Na^+)_1 \times (Cl^-)_1 = (Na^+)_2 \times (Cl^-)_2$$

On the right side, since there is no other ion and since electrical neutrality has to be maintained, the concentrations of Na^+_2 and Cl^-_2 must be equal. On the left, since some of the Na^+ is neutralized by R^-, the Na^+_1 is greater than Cl^-_1. This effect was described by Donnan, that the concentration of a diffusible positive ion is greater on the side of the semipermeable membrane where there is a non-diffusible negative ion. Conversely, the concentration of the diffusible Cl^-ion is greater on the side of the membrane which does not contain the nondiffusible negative ion (R^-).

This uneven distribution of the diffusible ions is on account of a non-diffusible ion on one side of the membrane.

Fig. 1-7

Membrane Hydrolysis: If there is a non-diffusible ion on one side of a semipermeable membrane, it can produce hydrolysis and a difference in pH between the two sides of the membrane. This is due to ionization of water and the H^+ and OH^- ions acting as diffusible ions.

(1)	(2)	
Na^+	Na^+	Due to the presence of the non-diffusible anion R^- on the left, when equilibrium is reached between the two sides, the diffusible anion OH^- gets more concentrated on the right (2) and the pH becomes more alkaline.
R^-	OH^-	
H^+	H^+	
OH^-		

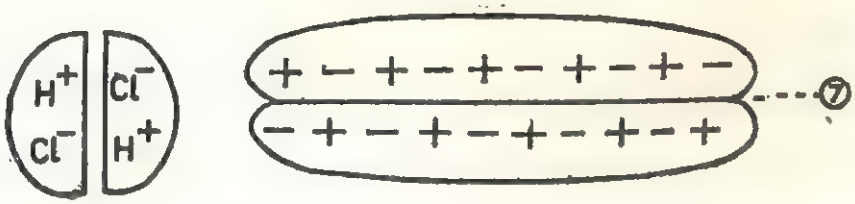
If the non-diffusible ion is a cation, the reverse will happen.

(1)	(2)	
R^+	H^+	Compartment (2) on the right will contain more of the H^+ ion than compartment (1) and is hence more acidic.
Cl^-	Cl^-	
H^+	OH^-	
OH^-		

In both instances, water is split up into H^+ and OH^- on account of the Donnan effect on the membrane. The phenomenon is called *Membrane Hydrolysis*.

INTERMOLECULAR FORCES

1. Dipole-dipole interaction: The positive end of a polar molecule will attract the negative end of a neighbouring polar molecule. eg. in HCl. Due to this interaction, polar molecules are held to each other more strongly than non-polar molecules of comparable size.



2. Van der Waals Forces: In the non-polar molecules, the average distribution of the charge may be symmetrical, but the electrons are in constant motion and at any given moment, due to the small distortion produced by the electron movement, a transient dipole

of a very small magnitude can be formed at one part of the molecule. This, in turn, will produce electronic disturbance in the neighbouring molecule, whereby the part of that molecule nearest to the disturbed moelcule develops an opposite charge at the point in proximity to the disturbed molecule. This process repeats itself with other molecules in a chain reaction and the molecules tend to arrange themselves such that the opposite charges are closer together.

The momentary (transitory) dipoles are constantly changing. So are the induced dipoles. But there is a net attraction between the molecules and they are kept together by these forces which are called van der Waals forces.

Additional Reading:

Cotton, F.A. and Wilkinson, G: Advanced Inorganic Chemistry, Wiley Eastern Pvt. Ltd., New Delhi, 1970.
Findlay, Alexandar: Introduction to Physical Chemistry, Longmans, London, 1960.
Morrison, R.T. and Boyd, R.N.: Organic Chemistry, Prentice-Hall India Pvt. Ltd., New Delhi, 1971.
Prescott Z. and Ridge, D: Organic Chemistry, University Tutorial Press Ltd., London, 1965.
West, E.S.: Biophysical Chemistry, Macmillan Co., N.Y., 1960.

ADDENDUM

i) The following prefixes are used in modern literature. They are used only to a limited extent in this text, but it is worthwhile knowing the terminology.

Units of mass and length:

1 dalton = mass of one hydrogen atom = 1.67×10^{-24} g
1 picogram = 1×10^{-12} g
1 nanometer (nm) = 1×10^{-9} m = 10 Å (Angstroms)
1 micrometer (μm) = 1×10^{-6} m = 10,000 Å

ii) *Prefixes and their abbrevations in common use:*

Value	*Prefix*	*Abbreviation*
10^{6}	mega	M
10^{3}	kilo	k
10^{-1}	deci	d
10^{-2}	centi	c
10^{-3}	milli	m
10^{-6}	micro	μ
10^{-9}	nano	n
10^{-12}	pico	p
10^{-15}	femto	f
10^{-18}	atto	a

iii) Concentrations like blood glucose and urea are expressed as milligrams per 100 ml in the text. In some of the modern literature they are expressed as millimols per litre. Concentration in millimols per litre can be obtained by multiplying the mg/100 ml with ten and dividing by molecular weight of that substance.

$$\text{eg. 90 mg\% of glucose} = \frac{90 \times 10}{180} = 5 \text{ millimols per litre.}$$

iv) *Chemical analysis of human body*

	per cent
Ether extractable material (lipid)	19.44
Water	55.13
Protein	18.62
*Ash	5.43

(*Ca=1.907% P=0.925%)

SOME RING STRUCTURES OF IMPORTANCE IN BIOCHEMISTRY

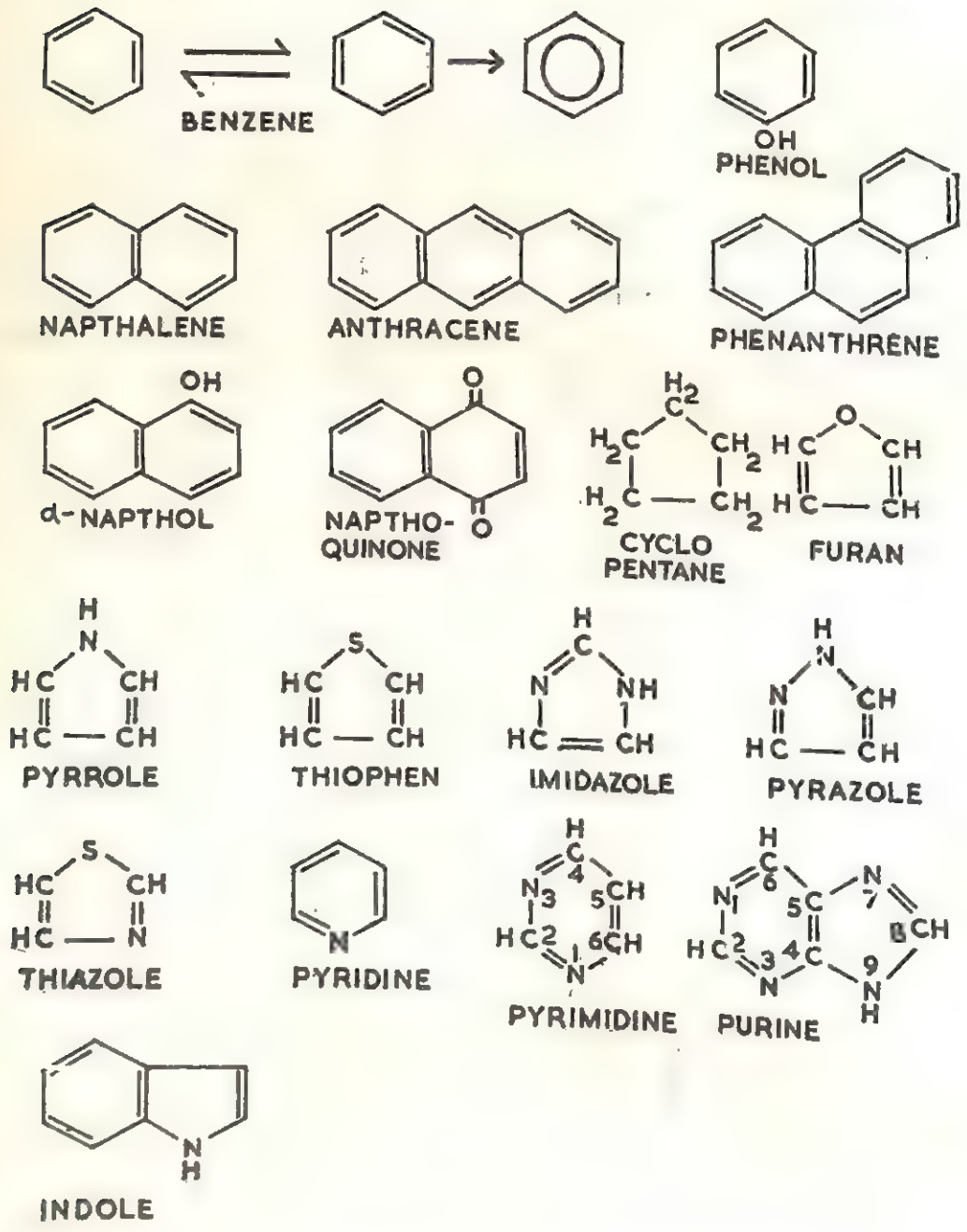


Fig. 1-8

1-A

BIOSTATISTICS

All scientific knowledge has to be evaluated statistically before sound conclusions can be drawn. A student of Biology or Medicine will come across statements made on the basis of statistical evaluation not only in biochemistry but in all other subjects as well. A brief introduction to the terminology used and an explanation of some of the simple calculations are therefore included in this chapter.

Observation: The basis of all learning process is sound observation by individual workers or organized institutions. The observer has to have a scientific outlook and must be capable of accurate observations based on well planned experiments or studies. Then only the observations can be relied upon.

Reasoning: A *notion,* a *premise* or an *assumption* is made based on a few observations initially, which may be just chance, unplanned observations (say three or four persons from a particular part of the country are taller than the local population). Then you start observing more carefully and watch several more people from that region. If all of them or most of them are taller than the local population, you come to the *conclusion* that people from that region are generally taller than people of your locality. The thought processes which enable you to draw a conclusion from your initial assumption are known as *reasoning.*

Logic: A set of rules are laid down for correct reasoning and are known as logic. Thus, you find dozens of crows in your garden and all of them are black. Wherever you go, all the crows you see are black. You therefore draw the conclusion that 'all crows are black.' This is correct reasoning. On the other hand, if you reason out that the black birds you see in your garden are crows; the black birds you see at every place are crows and therefore all black birds are crows, you are drawing conclusions based on inaccurate and insufficient observations whereby you have missed noticing the several other varieties of birds which are also black.

This type of reasoning based on individual observations (on the color of a few crows) and making a generalization (that all crows are black) is called *inductive reasoning.* It can be the other way also. You might have observed several monkeys and noticed that each one of them has a tail. When you next look at a monkey from a distance, even without looking at its hind portion, you can think: "All monkeys have tails. This is a monkey. So it must have a tail" This type of reasoning is called *deductive reasoning.*

The conclusions reached in either type of reasoning are called *inferences.* The statements– "All crows are black" or "All monkeys have tails"–are called *hypotheses.* If the

hypothesis is confirmed by several observations and preferably by several independent observers, it becomes a *theory.*

Frequency Distribution: If a constituent–say blood glucose–is estimated in a large number of people say 1,000 in the fasting condition, it will be found that individual values do not coincide and it will appear that there is much variation. But proper analysis of the values by statistical methods will show that there is a pattern even among the varying blood sugar values. They may all lie between 60 mg% to 100 mg%. Several persons may be having the same blood sugar%. For the sake of simplicity, if they are tabulated in increments of 5 mg% per block, the data will work out into a pattern like the one in the Table below.

Blood sugar mg%			*Number of individuals*
From		*To*	
60	–	65	20
66	–	70	60
71	–	75	150
76	–	80	270
81	–	85	270
86	–	90	150
91	–	95	60
96	–	100	20
			1000

A table such as the above showing the frequency (number of individual observations) of values falling in different intervals is called a *Frequency Distribution Table.*

The same data can also be represented graphically in the form of a *Frequency Distribution Curve* by plotting the mid values of the blood sugar levels on the X-axis and the number of individuals showing the value (frequency) on the Y-axis., and joining them by a smooth curve (see Fig. 1-9).

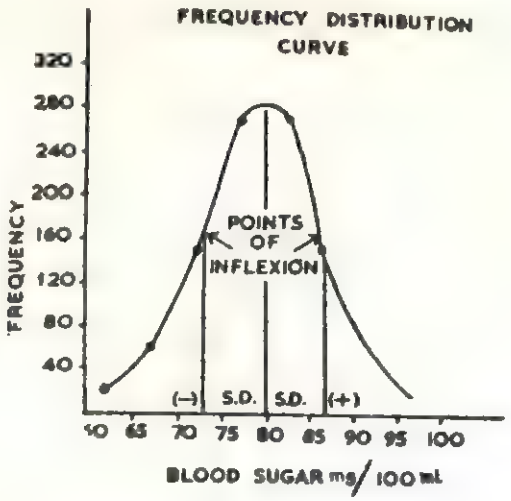


Fig. 1-9

A typical frequency distribution curve that we get for several cases is bell shaped and symmetrical. It is called a Normal or Gaussian distribution curve.

Arithmetic Mean: This is the simple average or mean of the sample observations and can be calculated by the formula

$$x = \frac{S(x)}{n}$$

Where x=arithmetic mean, S(x)=sum of all the values x_1, x_2, x_3 ..... x_n and n=the number of sample observations. In the Gaussian type of curve with perfect symmetry, a vertical line from the highest point of the curve will intersect the X-axis at the arithmetic mean.

Mode: The value that repeats itself most often in the series is called the mode. In the Gaussian type of curve, the mode will coincide with the arithmetic mean.

Median: The X-coordinate corresponding to the vertical line which divides the area under the curve into two equal halves is called the 'median'. In the symmetrical Gaussian curve, the median also coincides with the arithmetic mean.

In an assymmetric curve, the arithmetic mean, mode and median can all be different.

Standard Deviation of the normal Curve: The bell shaped curve will show an inflexion on the ascending as well as the descending limbs of the curve. If vertical lines are drawn from each of these points they will intersect the X-axis on either side of the mean at an equal distance from it. A large portion of the area under the normal curve has been included in the portion of the curve between the two points of inflexion. The distance between the mean and the point of inflexion on either side is equal to the Standard Deviation (S.D.) and is denoted by a ± sign prefixed to it to indicate that it extends on either side of the mean.

If another vertical line is drawn on either side of the mean at a distance equal to twice the S.D., most of the values in the distribution table would have been included in this part of the curve. In most cases, mean±S.D. will include 2/3 of the sample values and mean±2 S.D. will include more than 90% of values.

Scatter: The observed values are scattered on either side of the arithmetic mean. A measure of the distance between the smallest and the highest observed values is called the *Range*. When the range is specified (in this case it is 60-100 mg%) all individual values are contained in this interval.

Deviation: The Difference between any individual value and the mean is called the deviation from the mean. The deviation can be on the positive side or negative side. If the deviations of all the sample observations from the arithmetic mean are added, the total will be 'O' since the negative deviations cancel the positive ones.

Variance: If the deviations are squared, the negative sign is lost. The sum of the squares of all the deviations divided by the number of the deviations (which is same as the number of values) i.e. the mean of the squares of the deviations is called the Variance.

It is calculated by the formula:

where x = individual values x_1, x_2, x_3 etc.

$\frac{S(x-\bar{x})^2}{n}$. $\bar{x}$ = arithmetic mean

S = sum of and n = number of values.

The *Standard Deviation* is the square root of the Variance.

i.e. $\sqrt{\frac{S(x-\bar{x})^2}{n}}$

Where the number of values available for analysis is not high, a typical bell shaped Gaussian curve cannot be obtained. Hence it will not be possible to obtain the S.D. from such a curve. But the calculation of S.D. is still possible by the above method.

If the number of values in the sample is large enough say 20 or 30, then the arithmetic mean of the sample is likely to coincide with the arithmetic mean of the entire population. If the sample size is small, then the artithmetic mean calculated may not be the true mean of the entire population.

An estimate of the standard deviation in such cases i.e. when the sample size is small, is calculated using a slightly modified formula:

$$\text{S.D.} = \sqrt{\frac{S(x-\bar{x})^2}{n-1}}$$

(Note that the denominator inside the square root is 'n-1' instead of 'n)

Standard Error of the Mean: The means of samples of the same size selected from a given population need not always be same. The standard deviation of all such possible sample means is called standard error of the sample mean and is estimated by the formula:

$$e_{\bar{x}} = \frac{\text{S.D.}}{\sqrt{n}}$$

Where $e_{\bar{x}}$ is the standard error of the mean, S.D. is the estimated standard deviation of the individual measurements and n the number of individuals in the sample.

An example will make these concepts clear:

If the blood sugar in milligrams per 100 ml is estimated in the fasting condition in 10 normal individuals, the results and the calculation of the mean and standard deviation and standard error are shown on page 33.

Serial number	Blood sugar mg/100 ml	Deviation $(x_1-\bar{x}_1)$	(Deviation)^2 $(x_1-\bar{x}_1)^2$
1	66	−12	144
2	78	0	0
3	86	+8	64
4	72	−6	36
5	86	+8	64
6	65	−13	169
7	84	+6	36
8	75	−3	9
9	78	0	0
10	90	+12	144
Total	780	0	666

$$\therefore \text{Mean } (\bar{x}_1) = \frac{780}{10} = 78$$

$$\text{Variance} = \frac{666}{10} = 66.6 \left(S(x_1-\bar{x}_1)^2/n\right)$$

$$\text{Estimate of Standard Deviation} = \sqrt{\frac{S(x_1-\bar{x}_1)^2}{(n-1)}} = \sqrt{\frac{666}{9}} = 8.602$$

$$\text{Standard Error of the mean } \rho x_1 = \frac{\text{S.D.}}{\sqrt{n}} = \frac{8.602}{\sqrt{10}} = 2.710$$

Probability and Chance Differences: In biological studies, we meet with several variations in several components (say blood pressure, blood sugar, conduction time of an impulse etc) in response to disease, drugs or other stimuli, internal or external. It will be necessary to determine whether the observed change could have been by mere chance or is a real and *significant* change in response to the stimulus. If the probability "P" of the observed difference between two sets of results to occur by chance is 1 in 20(5% or 0.05 in 1) or less, then the observed change is said to be significant and it is indicated by $P \leq 0.05$. If the probability is 1 in 100(1% or 0.01 in 1) or less, then the observed change is highly siginificant and $P \leq 0.01$. The testing for the significance of a difference between two sets of data is done by what is known as the "t-test".

Procedure: If there are two sets of data, the arithmetic mean for each group is calculated and $\bar{x}_1$ and $\bar{x}_2$ are determined. The difference between the two means is calculated as $(\bar{x}_1 - \bar{x}_2)$ The estimate of the standard error of the mean differences of two sample means, if the samples are obtained independently is calculated as follows:

$$\text{ESTIMATE OF S.E.} = \sqrt{\frac{S(x_1-\bar{x}_1)^2 + S(x_2-\bar{x}_2)^2}{(n_1-1)+(n_2-1)} \left(\frac{1}{n_1}+\frac{1}{n_2}\right)} \quad \text{(A)}$$

where x_1 refers to first set of vales with mean $\bar{x}_1$
x_2 refers to second set of values with mean $\bar{x}_2$

and n_1 and n_2 are the numbers of individuals in each of the two sets. For simplifying further calculations, let us assume the samples to be of equal size i.e. $n_1=n_2$.

To take a concrete example, the blood sugar values of the normal group can be compared with that of a diabetic group. If the blood sugar in the fasting condition of 10 diabetic individuals is estimated and the results are tabulated as before:–

Serial number	Blood sugar mg/100 ml	Deviation $(x_2-\bar{x}_2)$	(Deviation)^2 $(x_2-\bar{x}_2)^2$
1	90	–28	784
2	140	+22	484
3	130	+12	144
4	100	–18	324
5	96	–22	484
6	110	–8	64
7	124	+6	36
8	116	–2	4
9	135	+17	289
10	139	+21	441
Total	1180	0	3054

Mean $(x_2)=1180/10=118$

Variance$=3054/10=305.4$

Estimate of Standard Deviation $=\sqrt{\frac{3054}{(10-1)}} = 18.42$

Estimate of Standard Error of the mean $= 18.42/\sqrt{10} = 5.825$

Tabulating the results for the two series:

Set	Number(n)	(n–1)	Mean Blood Sugar mg/100 ml	S $(x-\bar{x})^2$ (Deviation)^2 sum of
Normal	10	9	78	666
Diabetic	10	9	118	3054
Sum	20	18	Difference 40	Sum 3720

Estimate of the standard error of difference of sample means using formula 'A' is:

$$\sqrt{\frac{666 + 3054}{(10-1)+(10-1)}\left(\frac{1}{10}+\frac{1}{10}\right)} = 6{\cdot}429$$

$$\therefore t = \frac{\bar{x}_1 - \bar{x}_2}{\text{ESTIMATED S.E.}} = \frac{40}{6{\cdot}429} = 6{\cdot}223$$

Once 't' value is calculated, statistical tables are available (see table 1-3) which give the 'P' values for the different values of 't' and $(n_1 - 1) + (n_2 - 1)$. For the 't' value of 6.223 and $(n_1 - 1) + (n_2 - 1)$ of 18, 'P' is less than 0.01 ($P < 0.01$). Hence the blood sugar in this group of diabetics on the average shows siginificantly higher value from that of normals.

x°	P = ·9.	·8.	·7.	·6	·5	·4.	·3.	·2.	·1.	·05	·02	·01
1	·158	325	·510	·727	1·000	1 376	1·963	3·078	6·314	12·706	31·821	63·657
2	142	·289	·445	·617	·816	1·061	1·386	1·886	2·920	4·303	6·965	9·925
3	·137	·277	·424	·584	·765	·978	1·250	1·638	2·333	3·182	4·541	5·841
4	·134	·271	·414	·569	·741	·971	1·190	1·533	2·132	2·776	3·747	4·604
5	·132	·267	408	·559	·727	·920	1·156	1·476	2·015	2·571	3·365	4·032
6	·131	·265	·404	·553	·718	·906	1·134	1·440	1·943	2·447	3·143	3·707
7	·130	·263	·402	·549	·711	·896	1 119	1·415	1·895	2·365	2·998	3·499
8	·130	·262	·399	·546	·706	·889	1·108	1·397	1·860	2·306	2 896	3·355
9	·129	·261	·398	·543	·703	·883	1·100	1·383	1·833	2·262	2·821	3·250
10	·129	·260	·397	·542	·700	·879	1·093	1·372	1·812	2·228	2·764	3·169
11	·129	·260	·396	·540	·697	·876	1·088	1·363	1·796	2·201	2 718	3 106
12	·128	·259	·395	·539	·695	·873	1·083	1·356	1·782	2·179	2·681	3·055
13	·128	·259	·394	·538	·694	·870	1·079	1·350	1·771	2·160	2·650	3 012
14	·128	·258	·393	·537	·692	·868	1·076	1·345	1·761	2 145	2·624	2·977
15	·128	·258	·393	·536	·691	·866	1·074	1·341	1·753	2·131	2·602	2·947
16	·128	·258	·392	·535	·690	·865	1·071	1·337	1·746	2·120	2·583	2·921
17	·128	·257	·392	·534	·689	·863	1·069	1·333	1·740	2·110	2·567	2·898
18	·127	·257	·392	·534	·688	·862	1·067	1·330	1·734	2·101	2·552	2·878
19	·127	·257	·391	·533	·688	·861	1 066	1 328	1·729	2·093	2 539	2·861
20	·127	·257	·391	·533	·687	·860	1·064	1·325	1 725	2·086	2 528	2·845
21	·127	·257	·391	·532	·686	·859	1·063	1·323	1·721	2·080	2·518	2·831
22	·127	·256	·390	·532	·686	·858	1 061	1·321	1 717	2·074	2·508	2·819
23	·127	·256	·390	·532	·685	858	1·060	1·319	1·714	2 069	2 500	2 807
24	·127	·256	·390	·531	·685	·857	1·059	1·318	1·711	2·064	2·492	2·797
25	·127	·256	·390	·531	·684	·856	1·058	1·316	1 708	2·060	2 485	2 787
26	·127	·256	·390	·531	·684	·856	1·058	1·315	1·706	2·056	2·479	2·779
27	·127	·256	·389	·531	·684	·855	1·057	1·314	1·703	2·052	2·473	2·771
28	·127	·256	·389	·530	·683	855	1·056	1·313	1·701	2·048	2·467	2·763
29	·127	·256	·389	·530	·683	·854	1·055	1·311	1·699	2·045	2·462	2·756
30	·127	·256	·389	·530	·683	·854	1·055	1·310	1·697	2 042	2·457	2·750
∞	·12566	·25335	·38532	·52440	·67449	·84162	1·03643	1·28155	1·64485	1·95996	2·32634	2·57582

Table 1-3

References:

1. Bernstein, L. and Weatherall, M.: Statistics for Medical and other Biological Students, Livingstone, Edin & London, 1952.
2. Snedecor, G.W. and Cochran, W.G.: Statistical Methods, Iowa State College Press, Ames, Iowa, 1952.

2

CARBOHYDRATES

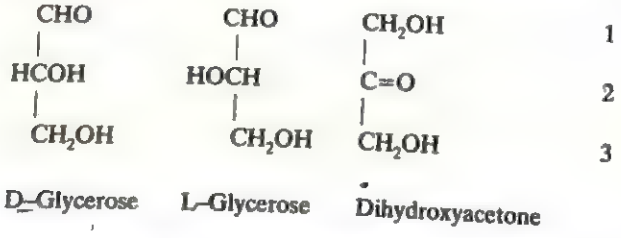
CARBOHYDRATES occur abundantly in nature. Cellulose of wood and paper, starches present in cereals, roots and tubers, cane sugar and milk sugar are all examples of carbohydrates. Animal tissues contain glycogen and body fluids contain glucose both of which are carbohydrates. Chemically they contain the elements carbon, hydrogen and oxygen, the latter two in the same ratio as in H_2O. Hence the name 'carbohydrate'. They are of the nature of polyhydroxy aldehydes or ketones or can be converted to such compounds on appropriate treatment. They are also called 'saccharides' ('sakcharon' in Greek meaning 'sugar'). The general formula, $C_n(H_2O)_n$ represents most of the carbohydrates.

Classification: Carbohydrates are classified into three groups: (i) Monosaccharides (ii) Oligosaccharides and (iii) Polysaccharides.

MONOSACCHARIDES OR SIMPLE SUGARS

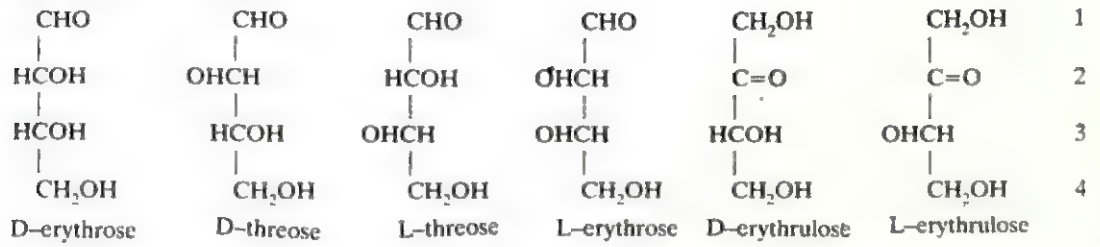
These are compounds with two to ten carbons per molecule (dioses to decoses). The more important ones have between three to six carbons (trioses, tetroses, pentoses and hexoses). Sugars which contain an aldehyde group are called aldoses. Those containing a keto group are called ketoses.

Trioses: Among the trioses with the aldehyde group (aldotrioses), since the carbon atom in the second position is an asymmetric one, two optically active forms, D–glycerose and L–glycerose, are possible. In the case of ketotriose there is no asymmetric carbon.



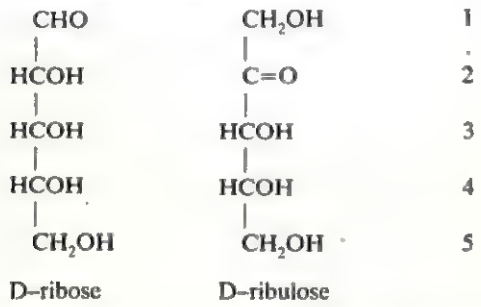
Tetroses: The chain length can be extended by reduction of the –CHO to a CHOH and addition of a new –CHO group in the case of aldose sugars. The last two carbons remain unchanged. All sugars having a configuration of H and OH in the last two carbons as in D–glycerose are called D–sugars and those having a configuration as in L–glycerose are called L–sugars, irrespective of their optical rotation. In fact, some of the D–sugars are levorotatory, (e.g. D–fructose has a specific rotation of –92°). The two aldotrioses can form four aldotetroses while the single ketotriose can give two ketotetroses. Their names and

structures are shown below:



While ketotriose is optically inactive, the ketotetroses are active on account of the asymmetric carbon atom in the third position and form the reference substances for the higher ketose sugars.

Pentoses: In a similar way each of the four aldotetroses can form two pentoses giving a total of eight aldopentoses and each of the ketotetroses can give two ketopentoses making a total of four ketopentoses. In general 2^n gives the number of optical isomers possible for a given sugar, where 'n' stands for the number of asymmetric carbons in the molecule. The structures of the more important pentoses are given below:



Some of the other pentoses are xylose, arabinose, etc.

Hexoses: Sixteen aldohexoses (2^4=16, due to presence of 4 asymmetric carbons) and eight ketohexoses (2^3=8, due to 3 asymmetric carbons) are possible. But only a few are of biologic importance.

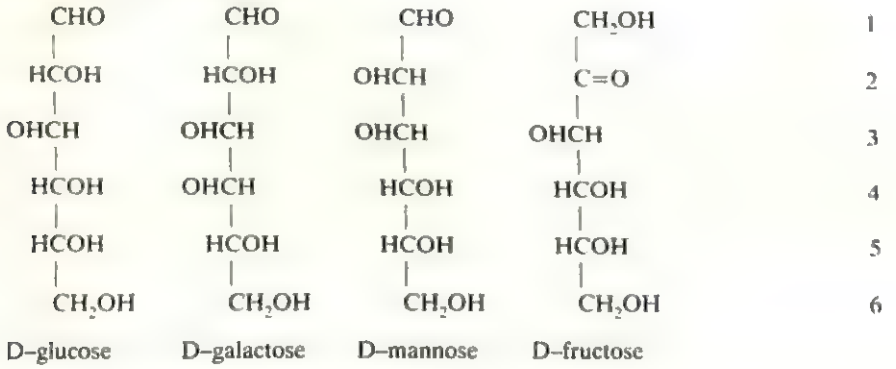
D–Glucose or Dextrose: Biologically, this is the most important sugar. It is the carbohydrate currency of the body. All carbohydrates are digested to form glucose before they can be absorbed into the blood stream and get transported to the different tissues in the body. It is a component of sucrose (cane sugar). maltose, lactose (milk sugar), starch, glycogen and other biologically important carbohydrates.

D–Fructose: This is present in fruit juices and honey. It is also a constituent of cane sugar and inulin. Though it is called D–fructose on account of the configuration of 'H' and 'OH' around the last but one carbon atom, it is actually levorotatory.

D–Galactose: This is a constituent of lactose or 'Milk sugar'.

D–Mannose: It is present in some plant products like gums and is a constituent of several glycoproteins.

Molecular structure: The straight chain formulae used below are convenient to write but do not represent the actual molecular structure of the sugars. The aldehyde or keto group of the sugar does not exist free as shown below and does not exhibit certain properties exhibited by free aldehyde or keto groups.



Of the L–sugars, L–fucose, L–rhamnose and L–sorbose are the important ones.

L–Rhamnose is 6–deoxy–L–mannose. L–Fucose is 6–deoxy–L–galactose. They are important components of bacterial cell walls.

Two sugars which differ from one another only in the configuration around one specific carbon are called 'epimers' of each other. Thus D–glucose and D–mannose are epimers with respect to C-2 and D–glucose and D–galactose are epimers with respect to C–4.

Schiff's Test: When SO_2 is passed through a solution of fuchsin, the pink color of the solution is decolorized. Addition of aldehyde will restore the color. But addition of sugars does not. They do not also form additive compounds with sodium bisulfite, whereas aldehydes do form such additive compounds. On treatment with methyl alcohol and hydrogen chloride, two isomeric methyl derivatives of glucose (glucosides) are obtained having different optical rotation indicating that the first carbon which has taken part in the formation of the glucosides is asymmetric.

Mutarotation: A freshly prepared solution of glucose crystallized from water or alcohol at low temperatures exhibits a specific rotation of + 112°. But a solution of glucose crystallized from water above 98° or from boiling pyridine has a specific rotation of +19° only. When either of the solutions is kept for some time, the rotation gradually changes to 52.5° and remains constant there. This is known as mutarotation and is explained by the existence of two optical isomers of glucose, alfa D–glucose with a specific rotation of + 112°, and beta D–glucoe with a specific rotation of +19°, the former having a configuration 'HCOH' in the first carbon, the latter having a configuration of 'HOCH' in the first carbon. This is made possible by the –CHO group taking on a 'H' from the fourth or fifth carbon and forming a ring structure instead of a straight chain. The first carbon has therefore become asymmetric and can exist in two isomeric forms. In solution, both forms exist in equilibrium with the chain form in the ratio of 2 parts beta form and 1 part alfa form and traces of chain form.

The reaction between the aldehyde group of C-1 with the alcoholic group of C–4 or C–5 results in the formation of a hemiacetal. Similarly, the reaction between the keto group of C–2 of fructose with the alcoholic group of C–5 results in the formation of a hemiketal.

The alfa and beta forms of glucose are called 'anomers' and the C–1, the anomeric carbon.

Depending upon whether the ring is formed between the first and fifth carbons or the first and the fourth carbons, a five membered ring called 'pyranose' (Haworth) or a four membered ring 'furanose' structure results, and the sugar is accordingly termed a pyranose sugar or a furanose sugar. The ring structures of glucose and fructose are shown in Fig. 2-1.

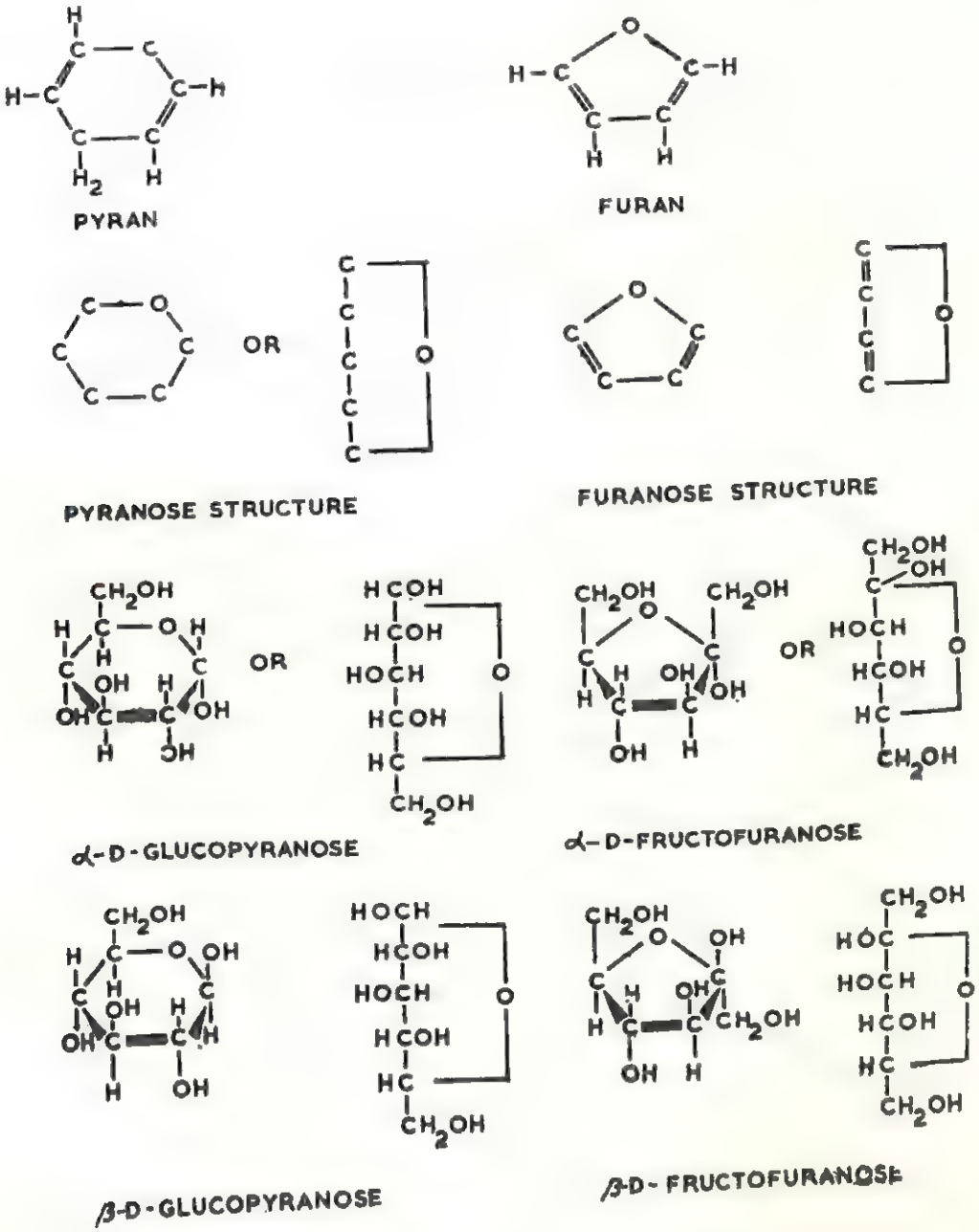


Fig. 2-1. Ring Structure of Monosaccharides

The ring has to be considered to be at right angles to the plane of the paper, the lower portion being nearer to the subject facing the paper. The 'H' and '–OH' project above or below the plane of the paper.

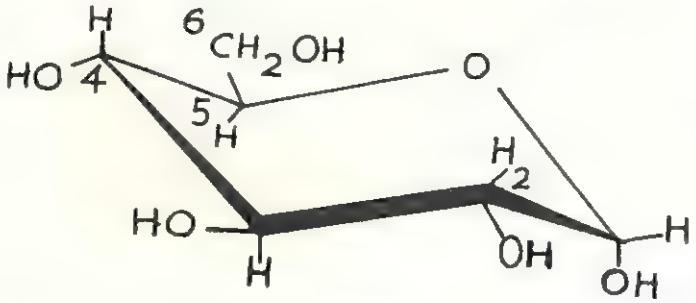


Fig. 2–2. Chair form of alfa - D glucose

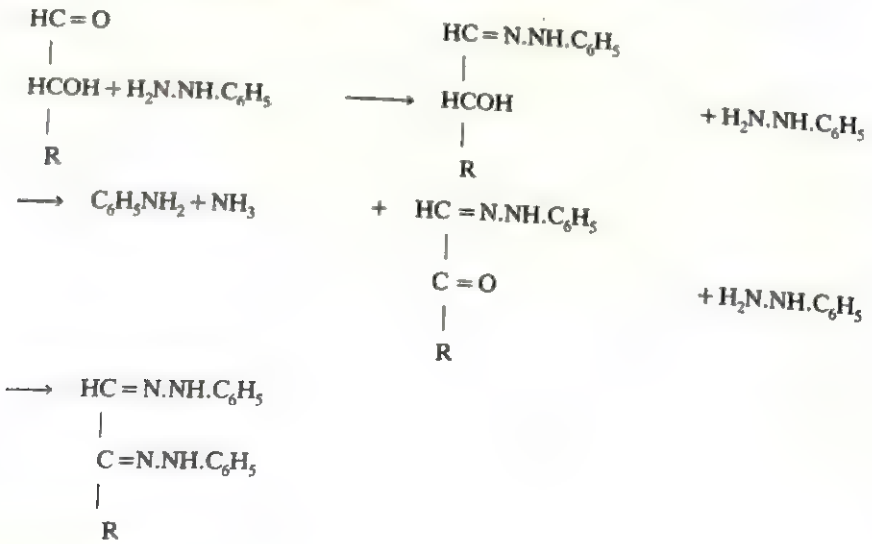
Even this configuration suggested by Haworth (1929) does not fully satisfy the requirements for stability of such molecules. The molecule will be more stable if the maximum number of hydroxy groups are in the equatorial plane, directed outwards, but almost in the same plane as the ring. This gives the molecule a structure more resembling a 'chair' or a 'boat'. Between the two forms, the 'chair' form is more stable (See Fig. 2-2).

PROPERTIES OF MONOSACCHARIDES

Physical: They are colorless, crystalline compounds, readily soluble in water and sweetish to taste. Their solutions are optically active and exhibit the phenomenon of mutarotation.

Chemical: **1.** Reactions characteristic of the aldehyde or keto group.

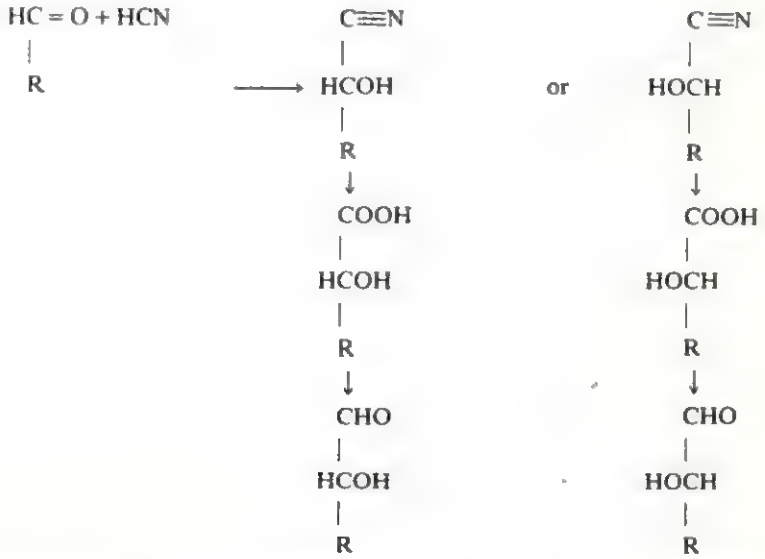
i. Formation of osazones wiyh phenyl hydrazine: The reaction involves the carbon containing the aldehyde or keto group and the carbon immediately next to it.



D–glucose phenyl osazone.

Since the first two carbons are involved in the reaction, hexsoses which have the same molecular structure in the remaining four carbons give the same osazones (e.g. glucose, mannose and fructose).

ii. Reaction with HCN to form cyanhydrins: The cyanhydrins can be hydrolyzed with suitable reagents to form two sugar acids, which on reduction can give two sugar molecules containing one carbon more than the starting point. This was the basis for the synthesis of sugars by Fisher and the nomenclature of D– and L– sugars.

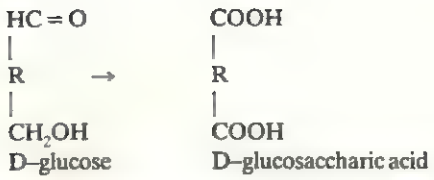


iii. Reduction of aldehyde or ketone group to form sugar alcohols: On treatment with sodium amalgam or with hydrogen under pressure in the presence of catalyst, the aldehyde or ketone group is reduced to an alcohol group.

iv. Oxidation to produce sugar acids.

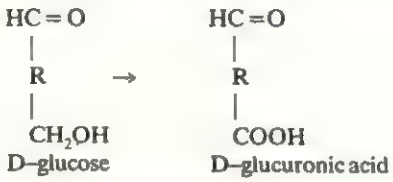
a) Mild oxidizing agents like bromine water oxidize the –CHO to a –COOH to form sugar acids.

b) Strong oxidizing agents like nitric acid, on the other hand, oxidize the aldehyde group as well as the primary alcoholic group to produce sugar acids called 'saccharic acids'.



Galactose forms an insoluble saccharic acid called 'mucic acid'. This property is used in the identification galactose.

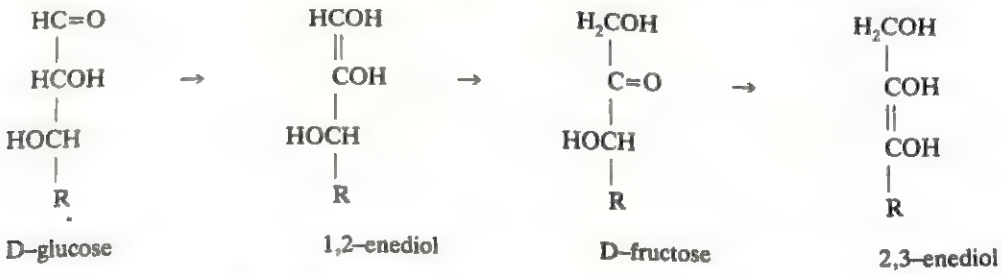
c) Uronic acids: If the primary alcoholic group (C_6 of hexose) is oxidized to a –COOH with the aldeyde group in C_1 unchanged, uronic acids are formed.



This is not possible under laboratory conditions since the more reactile –CHO group tends to be oxidized before the CH_2OH. In the living tissues, however, uronic acids are readily formed by enzyme action. They play an important role as components of structural material like chondroitin and mucoitin sulphuric acids and glycoproteins. They also play an important role in conjugation reactions whereby certain toxic substances like camphor are conjugated with glucuronic acid in the liver and excreted as less toxic substances. Some physiological and relatively insoluble substances like bile pigments and steroid hormones are also conjugated with glucuronic acid to render them more soluble and readily excretable through urine.

The L – epimer of glucuronic acid is known as iduronic acid.

v. Action of alkali upon sugars: Weak alkaline solutions of sugars undergo molecular change known as tautomerization whereby the 'H' atoms migrate from one carbon to another to form a mixture of enolic compounds.



The enediols can readily break down at the double bond to yield a complex mixture of aldehydes. This is why an alkaline medium is more fovourable for the reducing action of the sugars.

vi. Reducing action of sugars: In an alkaline medium, the aldehyde or keto group can reduce a number of substances like silver, bismuth, mercuric and cupric salts to metallic

silver, bismuth, mercury and cuprous hydroxide or oxide. Ferricyanide is reduced to ferrocyanide.

Simple laboratory tests for sugars usually employ the reduction of cupric salts to cuprous salts. Fehling's reagent contains cupric sulphate in a solution of NaOH and sodium potassium tartrate. While NaOH provides an alkaline medium, the purpose of sodium potassium tartrate is to keep the cupric hydroxide in solution by forming a complex with it. Benedict's reagent contains cupric sulfate in a solution of Na_2CO_3 (to provide an alkaline medium) and sodiam citrate (which helps to keep the cupric hydroxide in solution). This is the most commonly used reagent to test urine for reducing substances. A positive test with either of the reagents is indicated by the formation of a yellow precipitate of cuprous hydroxide or a brick-red precipitate of cuprous oxide. Barfoed's reagent is a solution of copper acetate in acetic acid and is used for distinguishing monosaccharides from reducting disaccharides. The acidic reaction of the reagent is not optimal for reduction, but the monosaccharides, being powerful reducing substances, will bring about a light reduction and produce a slight reddish precipitate. Disaccharides like lactose and maltose are less potent as reducing substances and fail to cause any reduction at all in the acid medium.

II. Reactions due to the alcoholic groups

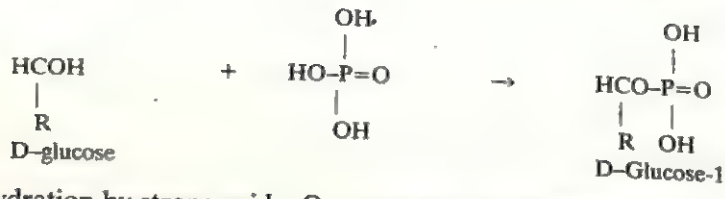
i. Formation of glycosides with alcohols. In the presence of HCL as catalyst, glycosides are formed with other alcohols. The first carbon takes part in this reaction and can form both alfa and beta glycosides. These compounds are of the nature of ethers formed between two substances having '–OH' groups.

α -D–glucose α -D–glucoside

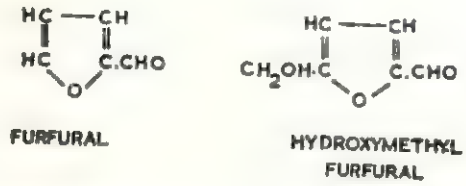
β-D–glucoside

A number of glycosides occur in nature, e.g. phlorhizin (glucose+phloretin) in rose bark; digitonin (4 galactose+xylose+digitogenin) in fox-glove leaves; amygdalin (2 glucose+2 mandelonitril) from bitter almonds and saponin (sugar+sapogenin) from soap wort. They are useful as medicaments.

ii. Formation of esters: The alcohol group may react with acids to form esters under suitable conditions. The phosphoric acid esters of sugars are important as intermediate products formed during metabolism in the body. Glucose-I-phosphate, glucose-6-phosphate, fructose-6-phosphate, fructose-1, 6-disphosphate and galactose-1-phosphate are some examples.



iii. Dehydration by strong acids: On treatment with concentrated sulfuric acid in cold or on heating with a strong solution of hydrochloric acid, neighbouring –OH groups are removed as H_2O and furfural is formed from pentose sugars. Hexoses form hydroxy methyl furfural. This reaction forms the basis for the Molisch's test, a general test for the identification of carbohydrates.



Sugar derivatives of biological importance

i. Amino sugars: Any of the –OH groups of the sugar can be replaced by an amino group. Usually the –OH of the second carbon is replaced. Glucosamine (2–amino D–glucose) and galactosamine (2–amino D–galactose) are examples. Glucosamine is an important constituent of mucopolysaccharides and mucoproteins such as hyaluronic acid, heparin and blood group substances. It is also present in the cell walls of the fungi and the shells of crustacea (lobster, crab, etc) as 'chitin'. Hence glucosamine is also called 'chitosamine'. Galactosamine occurs in sulfated mucopolysaccharides like chondroproteins, made up of chondroitin sulfuric acid and protein.

Muramic acid and Neuraminic acid:

These are components of polysaccharides of cell wall and are present as glycoproteins. They are nine carbon aminosugar derivatives. N–Acetyl derivatives of neuraminic acid are called *'silalic acids'*.

Neuraminic acid is formed by condensation of pyruvic acid with D-mannosamine.

ii. Deoxy sugars: If the 'o' of any of the secondary alcoholic groups 'HOCH' is absent, the sugar is called a deoxy sugar. 2-deoxy ribose ('o' from second carbon is absent) is an important derivative and is a constituent of deoxy-ribonucleic acid (DNA).

6-Deoxy L-galactose is L-fucose.

6-Deoxy L-mannose is L-rhamnose.

Oligosaccharides: This group includes substances containing two to ten monosaccharide units per molecule. The disaccharides containing two monosaccharides alone will be considered.

DISACCHARIDES

These are formed by union of two monosaccharides. They are united by linkage between the first carbon of one monosaccharide with the second or the fourth carbon of

another monosaccharide by what is known as the glycosidic linkage. The structures of the three biologically important sugars–maltose, lactose and sucrose are shown in Fig. 2–3.

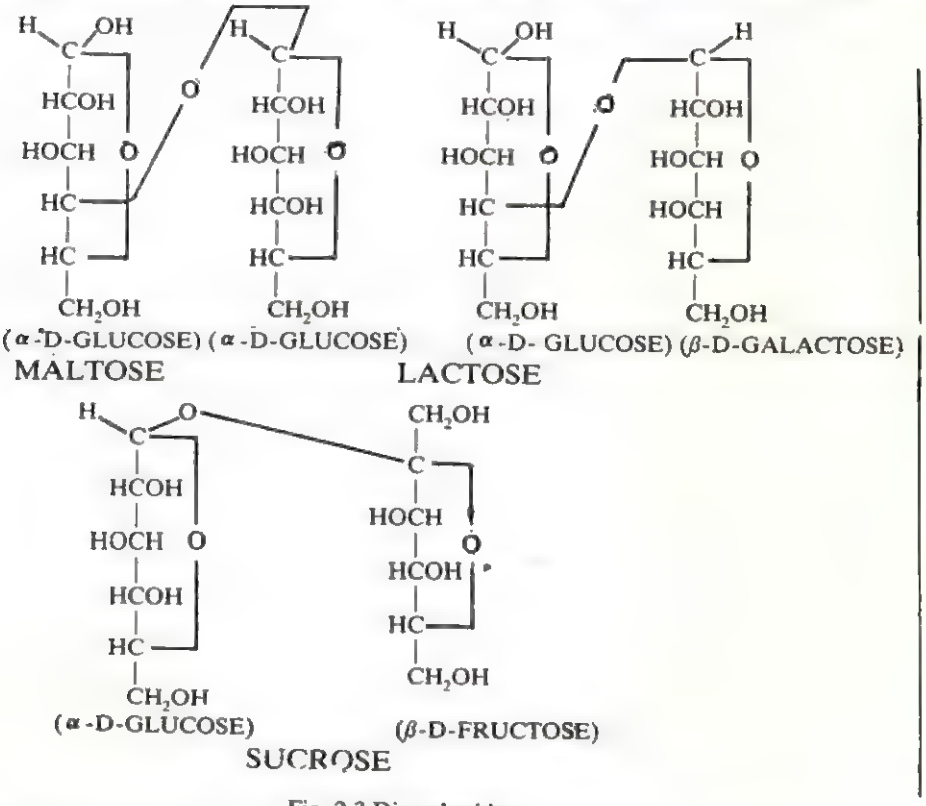


Fig. 2-3 Disaccharides

They are colorless crystalline substances readily soluble in water and sweetish to taste.

Maltose: This is made up of two alfa D–glucose units united by the 1, 4 –glycosidic linkage. Since one of the aldehyde groups remains free, it exhibits all the properties of the aldehyde group such as formation of osazones, reduction of cupric salts and others. Maltosazone crystals have a characteristic petal like appearance and a cluster of them looks like the sun-flower. Maltose is formed during the digestion of starches by enzymes or by dilute acids.

Lactose: This is made up of a molecule of alfa D–glucose united by 1,4–glycosidic linkage to a molecule of beta D–galactose. The aldehyde group of glucose remains free and exhibits the properties due to that group. Lactosazone crystals have a typical hedgehog shape and are readily identifiable. Lactose is present in milk (milk sugar) and can be hydrolyzed to glucose and galactose by the enzyme 'lactase' present in intestinal juice.

Sucrose: It is made up of one molecule of alfa D–glucose and one of beta D–fructose united by a glycosidic linkage between the aldehyde and keto groups (C_1 of glucose and C_2 of fructose). There is hence no free aldehyde or keto group in sucrose. It is a non-reducing sugar. It does not also form osazones. Solutions of sucrose exhibit a specific rotation of +62.5°. On hydrolysis with acid or with the specific intestinal enzyme 'sucrase', it is converted into an equimolar mixture of glucose and fructose whose specific activities are +52.5° and –92°. The mixture exhibits a net specific rotation of –19° The phenomenon

by which the dextrorotatory sucrose is converted to a levorotatory mixture of glucose and fructose is known as 'inversion'. Sucrose is also called the 'invert sugar' and the enzyme sucrase is also known as 'invertase'. Sucrose occurs widely in nature as cane sugar, beet sugar and in several ripe fruits.

Cellobiose (from cellulose) and gentiobiose are other disaccharides of glucose formed by β 1, 4– and β 1, 6– glycosidic linkages. Both are reducing sugars. Trehalose is a disaccharide formed by two glucose units combining by 1, 1– linkage. It is a non-reducing sugar. It is contained in the hemolymph of some insects.

Trisaccharides: Raffinose (galactose+glucose+fructose) is found in sugar beets. Melezitose (glucose+fructose+glucose) is found in the sap of some coniferous trees.

The following abbreviations are used for some of the carbohydrates:

Gal	..	D–Galactose	Gal N	..	D–Galactosamine
Glc	..	D–Glucose	Gal NAC	..	N–Acetyl–D–galactosamine
Man	..	D–Mannose			
Fru	..	D–Fructose	Glc N	..	D–Glucosamine
Glc UA	..	D–Glucuronate	Glc NAC	..	N–Acetyl–D–glucosamine
Ido UA	..	L–Iduronate			
Neu	...	D–Neuraminate	Fuc	..	L–Fucose
Neu NAC or NAN		N–Acetyl–D–neuraminate	Rha	...	L–Rhamnose
Sia	..	Silate, usually same as NAN			

POLYSACCHARIDES

Several monosaccharide molecules combine to form polysaccharides. They have high molecular weights and are only sparingly soluble in water in the cold. They form colloidal solutions when heated with water. They are not sweetish and do not exhibit any of the properties of aldehyde or keto groups.

Where the polysaccharide is made up of several units of one monosaccharide only, it is called a *'homopolysaccharide'*. eg. starch, glycogen, cellulose, dextrans, inulin and agar.

Where it contains more than one monosaccharide, it is called a *'heteropolysaccharide'* eg. pectins and mucopolysaccharides.

Starches: Occur in grains, seeds and tubers. Starch grains from individual sources have characteristic shapes and can be identified microscopically. They are polysaccharides of glucose.

Starch is made up of two types of polysaccharides - i. 'Amylose', soluble in hot water and ii. 'Amylopectin', insoluble in hot water. Amylose is a polymer of 200–1,000 alfa-D glucose molecules united by I, 4-glycosidic linkages. It dissolves in hot water and gives a blue color with iodine. Amylopectin is also a polymer consisting of hundreds of chains of alfa-D glucose molecules united by I, 4–glycosidic linkages which are joined together like the branches of a

tree, each chain attached to a neighbouring chain by a I, 6-glycosidic linkage between neighbouring glucose molecules of the two chains. The structure is somewhat similar to that of glycogen. There are several thousand glucose molecules in each amylopectin molecule. It forms a gel with hot water and gives a violet color with iodine.

The enzyme amylase from saliva or pancreatic juice can hydrolyze starch to smaller units called 'dextrins' and finally to maltose. The dextrins give a bluish or reddish color with iodine in the early stages of hydrolysis and later do not give any color at all though the molecules are still large. They are accordingly called amylodextrins, erythrodextrins and achrodextrins respectively. The hydrolysis can also be brought about by boiling with dilute acids.

Glycogen: This is also known as animal starch, since it is the main polysaccharide occurring in animal tissues, particularly in liver and muscle. Oysters and certain varieties of rice also contain glycogen. Like starch, this is also a polysaccharide made up of glucose units united by 1, 4–linkages and branches arising by 1, 6–linkages. But the branches occur at shorter intervals and give a more compact, tree like structure. The molecular weight is about 4,000,000. Glycogen gives a reddish brown tint with iodine. The enzymic hydrolysis and synthesis of glycogen are considered in detail in the chapter on carbohydrate metabolism.

Cellulose: It is the chief constituent of the woody fibrous portion of plant material and is the most abundant of carbohydrates in nature. It is made up of long chains of *beta* D–glucose molecules united by 1,4–linkages. There is no branching. The enzyme 'cellulase' can hydrolyze cellulose. But the enzyme is present only in bacteria. Hence cellulose is not utilizable by man, but it serves the function of increasing the bulk of the food, thus giving a sense of satiety. Undigested cellulose also adds to the bulk of the feces (roughage) and aids in its propulsion.

Cellulose is the main constituent of paper and cloth. It is also the basis for the manufacture of several synthetic fibres like rayon.

Dextrans: They are produced by yeasts and bacteria. They are highly branched polymers of glucose. The linear chains are formed by 1, 6–alfa glycosidic linkages, while the branching occurs by 1, 2–, I, 3– or I, 4–glycosidic linkages. They have the property of absorbing water and forming viscous, colloidal solutions with water. They are not metabolized by the tissues and are thus useful, when administered intravenously, in retaining water in circulation for long periods. Hence they are used as plasma substitutes for intravenous infusions. Dextrans formed by bacteria on the surface of teeth are an important component of dental plaque.

Bacterial dextrans produced are chemically cross-linked to form gels like *'sephadex'* which are widely used in biochemical separation procedures.

Inulin: It is a low molecular weight polysaccharide (M.W. 5,000), consisting of D–fructose units. It occurs in tubers of chicory, dahlia bulbs, onion and garlic. It is not utilizable by man. It is used in assessing the glomerular filtration rate (G.F.R.) in the study of kidney function.

Agar: Agar is a polysaccharide present in sea weeds. It is made up of sulfated galactose units. It dissolves in hot water and sets to a gel on cooling.

It is used as a culture medium for bacteria and also in the treatment of constipation. It is not utilized by man and hence adds to the bulk of the feces and helps in its propulsion.

Pectins: They are present in apple, lemon and other fruits. They form gels with sugar solutions. They are polysaccharides of galacturonic acid, galactose and the pentose sugar arabinose.

Chitin: This polysaccharide is present in the exo-skeleton of invertebrates like the crab, lobster and the insects. It is made up of acetylated glucosamine (chitosamine) units.

Proteoglycans (mucoproteins) and glycosaminoglycans (mucopolysaccharides)

Proteoglycans are polysaccharides combined with small amounts of protein (95% carbohydrate and 5% protein). The carbohydrate moieties are called glycosaminoglycans. They have a repeating disaccharide unit which is acetylated or sulfated.

The proteoglycans form about 30% of the dry weight of tissues and form the ground substance in the connective tissues. They are polyanionic substances with high molecular weights.

Some of the glycosaminoglycans are listed in table 2-1.

Table 2-1

Glycosaminoglycans

Name	Repeating disaccharide unit	Occurrence
1. Hyaluronic acid	D–glucuronic acid & D–glucosamine	Connective tissue, skin, vitreous humor, synovial fluid, cartilage.
2. Chondroitin sulfate	D–glucuronic acid & D–galactosamine	Cartilage, bone, cornea, skin, arterial wall.
3. Dermatan sulfate	D–glucuronic acid & L–ioduronic acid	Skin, heart valves, tendons, arterial wall.
4. Keratan sulfate	D–galactose & D–glucosamine	Cornea, cartilage, intervertebral disc.
5. Heparin	D–glucuronic acid or L–ioduronic acid & D–glucosamine	Mast cells in lung, liver, skin and intestinal mucosa.
6. Heparan sulfate	" "	Lung, arterial wall and most cell surfaces.

Functions

Being polyanionic, they attract and tightly bind cations like Ca^{++} (eg. in bone). They also take up Na^+ and K^+. Hyaluronic acid is highly viscous and helps as a lubricant. Proteoglycans act as molecular sieves by allowing small molecules to pass through but retaining molecules of the size of albumin and immunoglobulins.

Some of the proteins which are associated with the proteoglycans are:

Fibronectin – formed by fibroblasts
Laminin – formed by endothelial cells and
Chondronectin – formed by chondrocytes.

Blood group substances and bacterial polysaccharides are also glycosaminoglycans and are considered elsewhere.

References:

Florkin, M. and Stotz, E: Comprehensive Biochemistry, Carbohydrates, Sec. II Vol 5, Elsevier Amsterdam, 1963.
Haworth, W.N.: The Constitution of Sugars. Longmans, N.Y., 1929.
Pigman, W.: The Carbohydrates, Acad. Pr., N.Y., 1957.
Sharon, N.: Polysaccharides. Ann. Rev. Biochem: 35: 485-520, 1966.
Stacey, M. and Barker, S.A.: Carbohydrates of Living Tissues, D.Van Nostrand & Co., Princeton, N J., 1962

3

LIPIDS

THE lipids are organic substances insoluble in water but soluble in organic solvents like chloroform, ether and benzene; they are esters of fatty acids or are subtances capable of forming such esters and are utilizable by the living organism. They form important dietary constituents on account of their high calorific value and the fat soluble vitamins and the essential fatty acids contained in them. In the body they are present in the cytoplasm as well as the cell wall and are also in specialized areas in the body as depots of fat in which form energy is stored. Nervous tissues are particularly rich in lipids which appear to serve an important role in their function. The subcutaneous fat serves the role of insulating against atmospheric heat and cold and also helps in rounding off the contours of the body.

Classification: Lipids are classified as folows:

1. Simple lipids: (i) Neutral fats; (ii) Waxes.
2. Compound lipids: (i) Phospholipids; (ii) Glycolipids; (iii) Lipoproteins and others.
3. Derived lipids: (i) Fatty acids; (ii) Glycerol; (iii) Sterols and others. The chemistry of glycerol is not considered here.

Fatty acids

They contain the elements C, H, and O. Most of the naturally occurring fatty acids are straight chain derivatives and have an even number of carbon atoms. They may be saturated or unsaturated. A few are cyclic.

Saturated fatty acids: They can be represented by the general formula $CH_3(CH_2)_nCOOH$. The simplest is acetic acid (n= o). Some of the acids in the series are listed below:

		carbons
Acetic acid	CH_3COOH	2
Butyric acid	$CH_3(CH_2)_2COOH$	4
Caproic acid	$CH_3(CH_2)_4COOH$	6
Caprylic acid	$CH_3(CH_2)_6COOH$	8
Capric acid	$CH_3(CH_2)_8COOH$	10
Lauric acid	$CH_3(CH_2)_{10}COOH$	12
Myristic acid	$CH_3(CH_2)_{12}COOH$	14
Palmitic acid	$CH_3(CH_2)_{14}COOH$	16
Stearic acid	$CH_3(CH_2)_{16}COOH$	18
Arachidic acid	$CH_3(CH_2)_{18}COOH$	20
Lignoceric acid	$CH_3(CH_2)_{22}COOH$	24

Unsaturated fatty acids: They contain one or more double bonds. Some of the important members of this series are listed below:

Palmitoleic acid: A derivative of palmitic acid with a double bond between the 9th and 10th carbons. The carbons are counted from the carboxylic end.

Oleic acid: Derivative of stearic acid with a double bond between C9 and C10.

Elaidic acid is the trans-isomer of oleic acid. It is not present in naturally occurring fats, but is formed during hydrogenation of fats (eg. in Vanaspathi). Consumption of these hydrogenated fats may cause the appearance of elaidic acid in human fat also.

Linoleic acid: Also derived from stearic acid with two double bonds, one between C9 and C10 and a second one between C12 and C13.

Linolenic acid: Also from stearic acid with three double bonds between C9 and C10; C12 and C13; C15 and C16.

Arachidonic acid: Derivative of arachidic acid with four double bonds between C5 and C6; C8 and C9; C11 and C12 and C14 and C15.

Linoleic and linolenic acids are present in plant sources. Archidonic acid is present only in mammalian tissues. It is synthesized from linoleic acid. Prostaglandins are synthesized from archidonic acid.

Cyclic fatty acids: Chaulmoogric oil, once used in the treatment of leprosy, contains cyclic fatty acids called chaulmoogric and hydnocarpic acids.

Saturated monohydroxy acids: Cerebronic acid is a fatty acid containing 24 carbons and one hydroxy group.

Unsaturated monohydroxy acids: Ricinoleic acid belongs to this group. It has 18 carbons and occurs in castor oil.

Prostaglandins: These are unsaturated hydroxy acids with a five membered ring in a 20 carbon skeleton. The name 'Prostaglandin' was first given by von Euler in the 1930s to a lipid soluble acidic substance found in seminal plasma. Prostaglandins are derived from polyunsaturated fatty acids and have profound biological activities. They facilitate fertilization of the ovum by causing uterine and cervical movements that help the upward migration of the spermatozoa from vagina into cervix and uterus. They also exert general stimulant action on smooth muscle and are vasopressor in certain species (dog, rat, monkey and man) and vasodepressor in certain others (cat and rabbit). Some of them induce regression in the corpus luteum of sheep. Infertility in the male is found to be associated with low seminal prostaglandin levels in some cases. High levels are found in the amniotic fluid of women in whom premature abortion occurred.

Recent research has shown that these substances are not confined to the male genital tract. They can be detected in several tissues and are found to function as regulators of metabolism. Human seminal plasma contains over a dozen diffrent prostaglandins. Several more are contained in other tissues. S. Bergstrom and others established their

structure. They are cyclic compounds with twenty carbons and can be derived from arachidonic acid by the action of an enzyme complex - prostaglandin synthase. The eighth and twelfth carbons are joined to form a ring with carbons - C8 to C12. The remaining carbons extend from C8 and C12 as two arms. The parent ring structure is called prostanoic acid. Prostaglandins are classified into two groups mainly - E and F series. PGE_1, PGE_2 and PGE_3 belong to E group. $PGF_1\ \alpha$, $PGF_2\ \alpha$, and $PGF_3\ \alpha$, are of the F series. These are primary prostaglandins. The PGE series contain a keto group at C-9 and a-OH group at C-11. PGF series have -OH groups at both positions. The numerals following the letters indicate the presence and position of the double bonds in two arms. The α and β following the number in the F series indicate the orientation of the -OH group at C-9.

The structures of two of these, PGE_1, and $PGF_2\ \alpha$ are shown in Fig. 3-1 along with that of arachidonic acid.

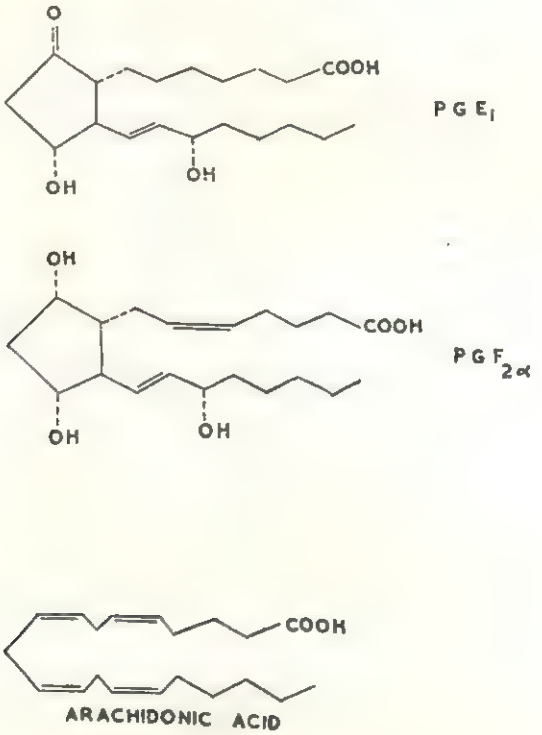


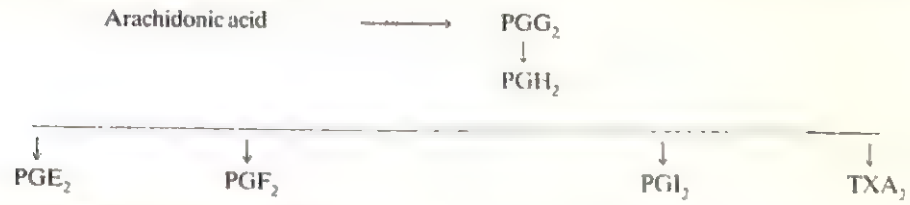
Fig. 3-1. Prostaglandins

The biological activity differs from one PG to another. Most of them, however, have some effect in lowering blood pressure and inducing smooth muscle contraction. PGE_1 appears to serve as a messenger between the hormone receptor on the cell surface and the adenylate cyclase enzyme more deeply located in the cell membrane (cytoplasmic surface). It is hence necessary for the hormonal stimulation of adenylate cyclase (see further in chapter 28 on ' Hormones'). On the other hand, prostaglandins seem to inhibit the adenylate cyclase of intestinal cells by binding to specific sites on the enzyme. They diminish gastric HCL secretion. Synthetic analogues of PGE_2 are being tried in the

treatment of peptic ulcer. The analogues have the advantage that they are not as rapidly destroyed as the natural prostaglandins. PGE_2 and $PGE_2\ \alpha$ are used clinically for inducting abortion.

Synthesis of prostaglandins seems to occur on stimulation by nerve impulse, hormones or drugs. The polyunsaturated fatty acids are first released by the action of phospholipases from phospholipids (mostly present in membranes) and then acted upon by the prostaglandin synthetase, a multienzyme complex. Aspirin, indomethacin and nonsteroidal anti-inflammatory drugs inhibit prostaglandin synthesis. The prostaglandins are inactivated or excreted soon after formation and hence have mainly local action at the site of production only. Inactivation is brought about by oxidation of the -OH group at C15.

Prostacyclin (PGI_2) and **thromboxane** (TXA_2) are formed from the same precursors as for prostaglandins but by different enzymes (prostacyclin synthetase and thromboxane synthetase). PGH_2 is the common precursor



These compounds have varying and sometimes even opposing actions on the smooth muscle of blood vessels, bronchi, gastrointestinal tract, and uterus. They also influence platelet aggregation, lipolysis, osteolysis, secretion of gastric HCL, pancreatic enzymes secretion and simulate the action of certain hormones like ACTH, TSH, LH, parathormone and gonadotropins.

For the use of prostaglandins as abortificants, see the chapter on 'Fertility and its Control'

Leukotrienes (LT)

These are conjugated trienes formed from arachidonic acid, in the leukocytes. They are potent constrictors of the bronchial musculature.

Physical Properties:

The lower fatty acids are liquids at room temprature; some of them are soluble in water and are steam volatile. The higher fatty acids are solids at room temperature and are insoluble in water but soluble in organic solvents already mentioned. Except acetic acid, all other fatty acids are lighter than water.

Gas-Liquid Chromatography of fatty acids:

The principle of separation is the same as in partition chromatography. The fatty acids in the mixture are first converted to their methyl esters to make them more volatile. An inert gas like nitrogen acts as the moving phase and carries the vaporized mixture of the methyl esters at high temperatures. The stationary liquid phase is made up of a polyester or silicone polymer with high melting point coated on particles of diatomaceous earth or on the inner surface of long, heated capillary tubes. The methyl esters of fatty acids partition themselves between the moving gas phase and the stationary liquid phase according to their gas-liquid partition coefficients and are carried by the gas phase at different rates and leave the column at different points or time. They can be detected by using suitable detector instruments which record the emergence of each fatty

acid ester as a separate peak. The area under each peak is a measure of the quantity of the particular fatty acid. A fraction of a milligram of the fatty acid mixture is sufficient for analysis by the gas liquid chromatography. The procedure can also be used to analyze mixtures of hydrocarbons, sterols and other compounds which are volatile at temperatures of about 350° C.

Chemical properties :

1. Formation of esters with alcohols : The esters of fatty acids with the trihydric alcohol, glycerol, are called neutral fats or triglycerides. Esters with some higher monohydroxy alcohols are called waxes.

$$RCOOH + C_2H_5OH \longrightarrow RCOOC_2H_5 + H_2O$$

2. Formation of soaps with alkalies.

$$RCOOH + NaOH \longrightarrow RCOONa + H_2O$$

The soaps of sodium and potassium are useful in daily life.

Sodium soaps are hard. Potassium soaps are soft but costly. To make the sodium soaps usable as toilet soaps, sodium carbonate or silicate is added in small amounts. This will make the soap lather even with hard water.

Shaving soaps are usually potassium soaps using coconut oil or palm oil as the source of fatty acids. To make the soap less alkaline and more smooth to the skin, excess of fatty acids are added.

Zinc stearate is a soft powder which is non-irritant to the skin and water repellent. It is commonly used in dusting powders. Calcium and magnesium soaps are insoluble in water and do not lather. Hard water which contains salts of calcium and magnesium is hence unsuitable for washing purposes.

Detergents: These are non-soap cleansing agents. They contain sodium salts of lauryl sulfuric acid, sulfated lauryl monoglyceride or salts of long chain fatty acids with quaternary ammonium as ingredients. Like soaps, they also are good wetting agents and emulsifiers. They have the advantage that they can lather equally well in hard water.

3. Reactions due to double bond of unsaturated fatty acids:

(i) Hydrogenation: Unsaturated fatty acids can, under suitable conditions of temperature and pressure and in the presence of catalysts, take up hydrogen at the double bond to form the corresponding saturated fatty acid.

$$\underset{\text{(Oleic acid)}}{RCH = CH \ldots COOH} + 2H \longrightarrow \underset{\text{(Stearic acid)}}{RCH_2CH_2 \ldots COOH}$$

(ii) Halogenation: The double bond can be also saturated by taking up Cl, Br or 1 under appropriate conditions.

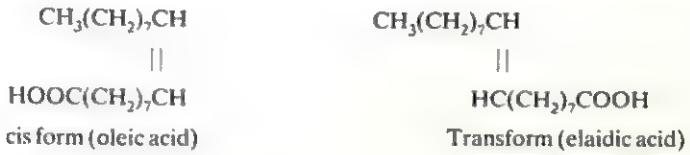
$$R\,CH = CH \ldots\ COOH + Br_2 \longrightarrow R\,CHBr\,CHBr \ldots\ COOH$$

(iii) Oxidation at the double bond: The carbons at the double bond are readily oxidizable. A number of intermediate compounds like hydroxy acids, aldehydes and peroxides are formed and finally, the fatty acid is broken down at the double bond to form a smaller fatty acid and a dicarboxylic acid.

$$RCH = CH \ldots\ COOH + O \longrightarrow RCOOH + HOOC \ldots\ldots COOH$$

Isomerism on account of the double bond: Isomerism is possible by varying the position of the double bond in the fatty acid molecule. The natural isomers and the positions of the

double bonds in them have been mentioned. In addition, a second type of isomerism is possible by changing the configuration of the groups on either side of the double bond. The eighteen carbon fatty acid with a double bond between C9 and C10 can exist in two forms.



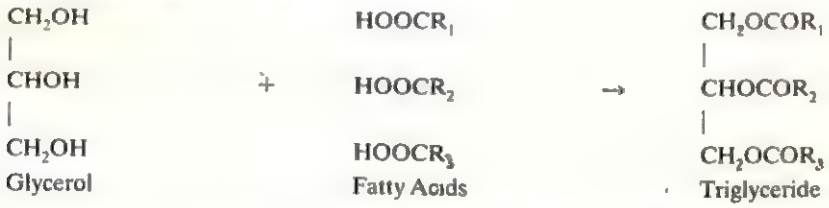
They are called cis-trans isomers. The naturally occurring fatty acids are the cis isomers. The trans isomers are not utilized by the organism.

With this basic knowledge of the chemistry of fatty acids, the chemistry of the lipids can now be considered.

SIMPLE LIPIDS

1. Neutral fats:

These are the fatty acid esters of the trihydric alcohol, glycerol. They are also known as glycerides. Some are solids at ordinary temperatures, while others are liquids. The latter are sometimes referred to as 'oils'.



Glycerol is optically inactive. But when it is esterified by two different fatty acids in positions 1 and 3, the carbon in position 2 becomes asymmetric and may be considered to be derived from L-glyceraldehyde.

R_1, R_2 and R_3 may be same or different fatty acids. Depending on the fatty acids present, they are called tripalmitin, tristearin, palmito-oleio-stearin, etc.

Physical properties:

Neutral fats are colorless, odorless, tasteless substances. The color and taste of some of the naturally occurring fats is due to extraneous substances (e.g. butter contains carotene). They are insoluble in water, but soluble in organic solvents. They have well defined melting points and solidifying points, the latter temperatures always a few degrees lower. They have low specific gravity and float on water. Oils spread on water to form thin monomolecular layers.

Chemical properties:

1. *Hydrolysis:* Heating with superheated steam or boiling with acids or alkalies will produce hydrolysis of the neutral fat into glycerol and fatty acids. In case alkali has been used for the hydrolysis, the fatty acids liberated will combine with the base to form soaps.

2. *Additive reactions:* The unsaturated fatty acids present in neutral fat will exhibit all the additive reactions (hydrogenation, halogenation). Oils which are liquid at ordinary temperatures on hydrogenation become solidified. This is the basis for the vanaspathi manufacture, where inedible and cheap oils like cotton seed oil are hydrogenated and converted to edible fat.

3. *Oxidation:* Fats very rich in unsaturated fatty acids such as linseed oil undergo spontaneous oxidation at the double bond forming aldehydes, ketones and resins which form thin transparent coating on the surfaces to which the oil is applied. These are called drying oils and are used in the manufacture of paints and varnishes.

4. *Rancidity:* Naturally occurring fats, particularly those from animal sources, are contaminated with enzymes like lipase. The action of the enzymes and also atmospheric moisture and temperature bring about partial hydrolysis of the fat and some degree of oxidation of the unsaturated fatty acids at the double bond. The fats will develop a characteristic taste and odor. The process is called rancidity and the fat is said to have become rancid. Vegetable fats contain substances like vitamin E, phenols, hydroquinone, tannins and others which are antioxidants and therefore prevent development of rancidity. Hence vegetable fats preserve for longer periods than animal fats.

Characterization of fats: Fats are characterized and their purity or otherwise assessed by determining certain chemical constants for individual fats.

1. *Saponification number:* Number of milligrams of KOH required to saponify the free and combined fatty acids in 1 gram of a given fat is its saponification number. A high saponification number indicates that the fat is made up of low molecular weight fatty acids and vice versa.

2. *Iodine number:* The number of grams of iodine required to saturate 100 grams of a given fat is defined as its iodine number. Since iodine is taken up by the double bonds, a high iodine number indicates a high degree of unsaturation of the fatty acids in the fat.

3. *Acid number:* Number of milligrams of KOH required to neutralize the free fatty acids in a gram of fat is known as the acid number. The acid number indicates the degree of rancidity of the given fat.

4. *Reichert-Meissl number:* The number of millilitres of 0.1 N alkali required to neutralize the volatile fatty acids (separated by saponification, acidification and steam distillation of the fat) contained in 5 grams of the fat is the Reichert-Meissl number.

The constants for some of the common fats are listed in table 3-1.

TABLE 3-1

Source	*Acid No.*	*Reichert Meissl No.*	*Saponification No.*	*Iodine No.*
Human fat ...	...	0.40	196	65
Beef fat ...	0.25	...	198	40
Butter fat ...	0.40	26.0	220	27
Linseed oil ...	2.2	1.0	192	190
Coconut oil ...	1.5	7.0	258	8

(The figures indicate only approximate averages).

WAXES

They are of the nature of insect secretions or protective coating on animal furs and leaves. Chemically they are esters of higher fatty acids with higher monohydroxy alcohols. Free fatty acids, alcohols and some hydrocarbons are also present mixed with the ester. A few of the waxes are listed below:

Bees wax: Palmitic acid ester of myricyl alcohol ($C_{30}H_{61}OH$).

Lanoline or wool fat: Palmitic, oleic or stearic acid ester of 'cholesterol', a complex structure which will be dealt with in detail under 'sterols'. Lanoline is useful in the manufacture of cosmetic creams, ointments, etc., since it closely resembles sebaceous secretion.

Spermaceti: Palmitic acid ester of cetyl alcohol ($C_{16}H_{33}OH$). It is an oil from the head of the sperm whale and is useful in the manufacture of polishes, ointments, candles, etc.

COMPOUND LIPIDS

Besides fatty acid and alcohol, they also contain other groupings.

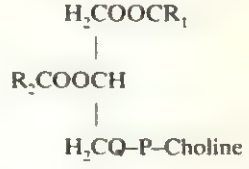
1. Phospholipids

Phospholipids are insoluble in dry acetone. They are present in abundance in brain and nervous tissues. Varying amounts are present in every living cell of plant or animal origin. They are present both in cytoplasm as well as cell membranes and serve important functions in both cell activity and cell permeability. They are of importance in insulating the nerve impulse (like the rubber or plastic covering around an electric wire) from the surrounding structures and in channelling out the enzymes into distinct groups. They also form important intermediate substances in the transport of lipids from and to the liver. They are made up of fatty acid, glycerol or other alcohol, nitrogenous base and phosphoric acid.

Classification: Phospholipids are classified differently by different authors. A recent classification (Celmer and Carter) is given below and is based on the alcohol moiety of the phospholipid.

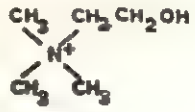
A. Glycerophosphatides: Glycerol is the alcohol in this group. This includes lecithins, cephalins (phosphatidyl ethanolamine), phosphatidyl serine, plasmalogens and diphosphatidyl glycerols.

(i) Lecithins: They are widely distributed in the body, but particularly rich in the liver. They are also present in plasma. They contain glycerol, fatty acid, phosphoric acid and a quaternary base 'choline'. The structure is shown below:



The structure shown is that of an alpha lecithin. In beta lecithin, the two fatty acids occupy the alpha positions while the base and phosphoric acid occupy the beta position. A large number of lecithins exist differing in their fatty acid moiety.

The base 'choline' which has the following structure is itself a very important substance in the prevention of accumulation of abnormal amounts of fat in the liver (lipotropic action of choline) and also as a constituent of acetylcholine, so important in the transmission of nerve impulses.



CHOLINE

Snake venom owes part of its toxicity to the presence of lecithinases – enzymes which hydrolyze the lecithins.

Lecithins lower the surface tension of water and aid in emulsification of lipid-water mixtures, a prerequisite in the digestion as well as absorption of lipids from the gastrointestinal tract. In the plasma, they serve the very useful function of keeping cholesterol and its ester in the dissolved state. If the base, choline, is removed, the resulting structure is called 'phosphatidic acid'.

(ii) Phosphatidyl ethanolamines (cephalins): They are structurally similar to lecithins except that the base is 'ethanolamine' ($NH_2CH_2CH_2OH$). They occur together with lecithins and are particularly concentrated in the brain. They are also present in the erythrocyte.

(iii) Phosphatidyl serine: Similar in structure to lecithins, but the base is an amino acid, serine ($OHCH_2CHNH_2COOH$). Occurrence and functions are also similar.

(iv) Plasmalogens: They form about 10% of the total phosphatides of muscle and brain. One of the fatty acids is replaced by a long chain aliphatic aldehyde. The aldehyde is in the enolic form (R CH = CH OH) and combines with the alfa carbon of glycerol by an ether linkage to form $CH_2OCH = CHR$. The remaining two carbons of glycerol have similar structure as in cephalins. The base is choline or ethanolamine.

(v) Diphosphatidyl glycerols: These are two moleucles of phosphatidic acid combined by a bridge of glycerol. Cardiolipin, initially isolated from heart muscle, is an example. It forms the basis for a serological test for the diagnosis of syphilis.

B. Phosphoinositides: In these the cyclic hexahydric alcohol 'inositol' replaces the base. Depending on whether there is only one phosphate (in alpha position) attached to inositol (in which case there are two fatty acids attached to the other two carbons) or two phosphates in the two alpha positions joined together by the inositol as bridge (in which case there is only one fatty acid esterifying the beta position), they are called a 'monophosphoinositide' or diphosphoinositide'.

C. Phosphosphingosides or sphingolipids: In this group of substances the trihydroxy alcohol, glycerol, is replaced by a complex amino alcohol, 'sphingol'. Fatty acid, choline and phosphoric acid are the other constituents. The structures of sphingol and sphingomyelin are given below.

Sphingomyelins are present in large amounts in brain and nervous tissues and only very small amounts are present in other tissues. In a disease called Niemann-Pick's disease large amounts of sphingomyelins accumulate in the spleen and the liver besides brain due to a metabolic defect.

2. Glycolipids, glycosphingosides or cerebrosides

These contain sphingol, a carbohydrate–galactose, and fatty acid. They do not contain phosphoric acid. Hence they are not phospholipids, but are called galactolipids or glycolipids. They are present in large amounts in the white matter of the brain and in the myelin sheathes of nerves. In Gaucher's disease they accumulate in large amounts in the liver and spleen.

Gangliosides: They constitute 6% of total lipids in gray matter. They are glycosphingolipids. The carbohydrate moiety contains one or more residues of sialic acid. In human brain, the gangliosides contain N-acetylneuraminic acid as the sialic acid component. It is made up of a molecule of N-acetyl, D-mannosamine combined with a molecule of pyruvic acid. The glycosphingolipids have important functions. As constituents of cell membranes, they are responsible for the blood group specificity and tissue and organ specificity. They are also responsible for tissue immunity and for cell-cell recognition sites. Gangliosides are particularly abundant in nerve endings. They may function in transmission of nerve impulses across synapses. They are also present in the receptor sites for acetylcholine and other neurotransmitter substances.

Gangliosides accumulate in brain in Tay-Sach's disease due to genetic lack of the enzyme required for its degradation.

Terpenes: They are polymers of the five-carbon hydrocarbon *"Isoprene"*.The side chains in vitamins A, E, and K, beta carotene and squalene are examples of terpenes. Natural rubber is a polyterpene containing hundreds of isoprene units in regular linear order.

Lipo-proteins will be considered under 'Proteins'.

DERIVED LIPIDS

This group consists of 1) Fatty acids and 2) Sterols. The chemistry of fatty acids had been already considered.

Sterols: These are derivatives of a complex ring system called 'cyclopentanoperhydrophenanthrene' ring system. The meaning of the name and the method of naming the individual compounds of the system as A, B, C, D rings and numbering of the seventeen positions in the ring system are depicted in Fig. 3-2.

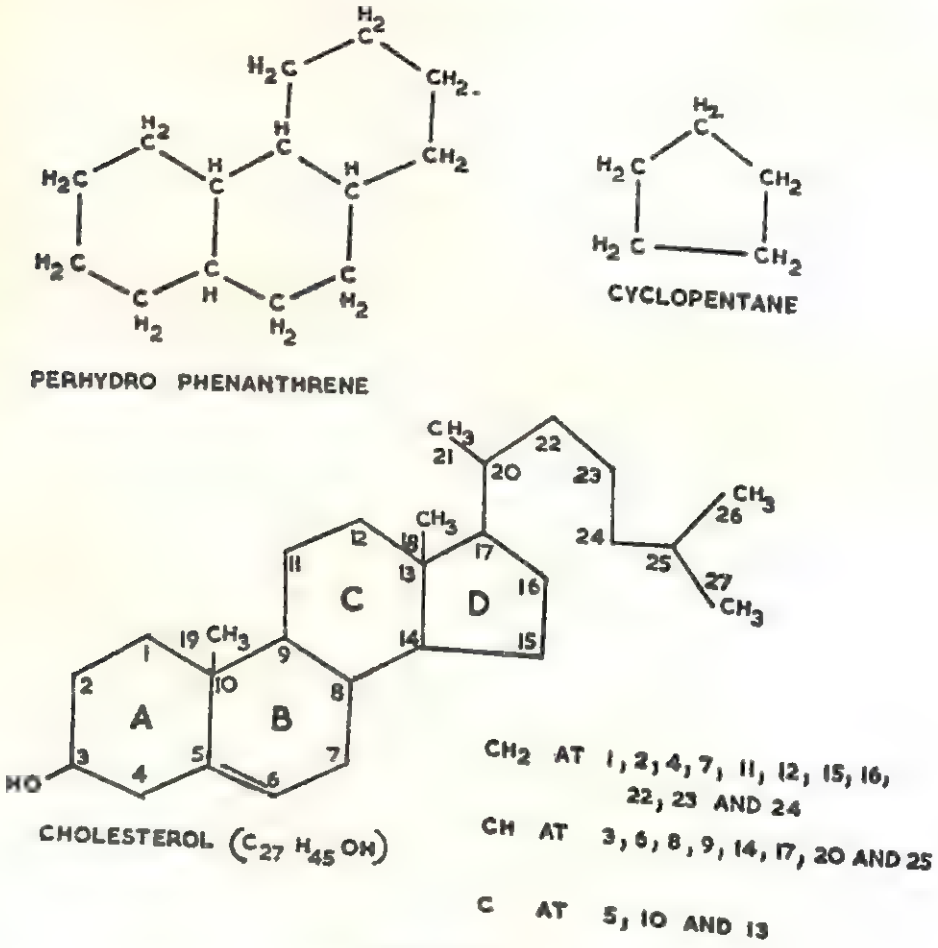


Fig. 3-2. Cholesterol

Cholesterol, ergosterol, bile acids, sex hormones, adrenal cortical hormones and the D-vitamins are some of the important sterol derivatives. The term 'sterol' means 'solid alcohol'.

Cholesterol: Since it was first isolated from gall stones, it was called cholesterol which means 'solid alcohol from bile'. Brain, nervous tissues, adrenal glands and egg yolk are rich sources. White matter contains as much as 14%, gray matter 5%, spinal cord 12% and liver about 1% cholesterol. It has a molecular formula $C_{27}H_{45}OH$ and has the structure shown in Fig. 3-2.

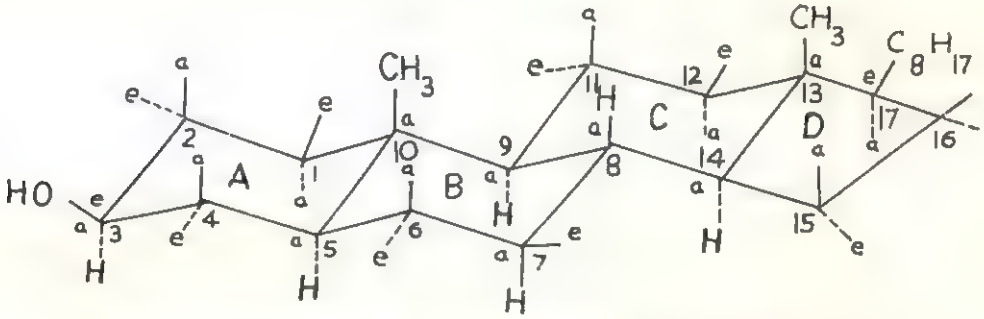
Saturation of the double bond between C5 and C6 will give dihydrocholesterol.

Coprosterol is the cis isomer of dihydrocholesterol and is found in feces.

While the ring structure shown in Fig. 3-2 is in common use, the three dimensional structure of the steroid molecule is more correctly represented if the rings are replaced by chair or boat forms. Between the two, the chair form is more stable.

Further, the CH_3 in position 10 is considered to be in the beta position, *i.e.*, projects above the plane of paper. If H in position 5 is in the same plane as the CH_3, the rings are in the cis-configuration and the steroid is said to belong to the beta series, and the bond linking H is shown as an unbroken line.

If the H in position 5 is in the opposite plane to that of CH_3 (*i.e.*, below the plane of the paper), the bond is represented by a broken line and the steroid belongs to the alfa series. Rings A and B are in the trans position to each other. These concepts and structures are illustrated in Fig. 3-3 for *cholestanol* (in which the double bond of cholesterol between C5 and C6 is saturated)



CONFORMATIONAL STRUCTURE OF CHOLESTANOL

Fig. 3-3

Cholesterol belongs to the alfa series. The H at position 5 is in trans position to the CH_3 at position 10. The OH at position 3 is however in cis relationship with the CH_3 at position 10. It has a double bond between C5 and C6, in the ring B.

Properties of cholesterol: It is a white crystalline substance showing the usual solubility properties of the lipids. The crystals are rhombic plates with one of the angles broken. It has a melting point of 149°.

It is usually prepared in the laboratory by extraction from brain or spinal cord with acetone. It is a poor conductor of electricity and functions probably as an insulating mechanism for the nerve impulses. As a precursor of bile salts, steroid hormones and vitamin D_3 it is of great significance. It is present in blood to the extent of 150–250 mg. per 100 ml. and its variations are of considerable clinical significance.

Chemical properties: The double bond can be saturated by addition of hydrogen to form the dihydroderivative. It can also be halogenated. The OH group in position 3 can be esterified with fatty acids to form cholesterol esters. Three fourths of the cholesterol of plasma exists as ester. Lanoline, the cholesterol ester, has been already considered under waxes. The OH

combines with digitonin to form an insoluble digitonide. This property is used to separate cholesterol from its esters which do not react with digitonin.

Color reactions

i) Salkowski reaction: When a solution of cholesterol in chloroform is shaken with an equal volume of concentrated sulfuric acid and the layers are allowed to separate, the chloroform layer turns red and the acid layer shows a greenish fluorescence.

ii) Lieberman-Burchard reaction: To a solution of cholesterol in chloroform, if a few drops each of acetic anhydride and conc. sulfuric acid are added, a rose-red color develops and rapidly changes to blue and finally green. A modified reagent containing ferric chloride, glacial acetic acid and sulfuric acid gives a purple color.

OTHER STEROLS

Ergosterol: ($C_{28}H_{43}OH$): Present in fungi, yeast and other vegetable matter. Besides differing in the side chain from position 17, it has also an additional double bond between C7 and C8. This is the precursor for vitamin D_2 (calciferol) while 7-dehydrocholesterol is the precursor for vitamin D_3.

Bile acids: The parent structure is 'cholanic acid'. The side chain at position 17 is oxidized to end in a COOH. Three different bile acids occur in nature. They differ only in the number of ring carbons oxidized to form an OH.

3-hydroxy cholanic acid is lithocholic acid.
3, 12-dihydroxy cholanic acid is desoxycholic acid.
3, 7, 12-trihydroxy cholanic acid is cholic acid.

They are conjugated in the liver with 'glycine' or 'taurine' to form 'glycocholic' or 'taurocholic' acids which combine with sodium to form the bile salts. The bile salts have a remarkable ability to lower surface tension and thus help in emulsification of fats.

Sex hormones and adrenal cortical hormones have also a steroid structure and will be discussed at the appropriate place.

References:

Bloor, W.R.: Biochemistry of the Fatty Acids and their compounds, the Lipids, Reinhold, N.Y., 1943.
Brady, R.D. and Trams, E.G.: The Chemistry of Lipids. Ann. Rev. Biochem., 33:75; 1964.
Carter H.E. et al: Glycolipids., Ann. Rev. Biochem. 34:109, 1965.
Celmer, W.D. and Carter, H.E.: Chemistry of Phosphatides and Cerebrosides., Physiol. Rev. 32: 167, 1952.
Von Euler, and Eliasson. R.: Prostaglandins., Acad. Pr., N.Y., 1967.
Fieser L.F. and Fieser M.: Steroids., Reinhold, N.Y., 1959.
Florkin, M. and Stotz, E.H. (Eds.): Comprehensive Biochemistry, Vol. 6, Lipids, Amino Acids and Related Compounds. Vol. 10, Sterols, Bile Acids and Steroids. Elsevier, Amsterdam, 1965 & 1963.
Hanahan, D.J. and Thomson G.A. Jr.: Complex Lipids. Ann. rev. Biochem., 32: 215, 1963.
Hilditch, T.P. and Williams. P.N.: The Chemical Constitution of Natural Fats, 4th ed., Chapman & Hall, London, 1964.
Khan, R.H. and Lands, W.E.M.: Prostaglandins and Cyclic AMP., Acad. Pr., N.Y., 1973.
Wittcoff, H.: The Phosphatides., Reinhold, N.Y., 1951.

4

PROTEINS

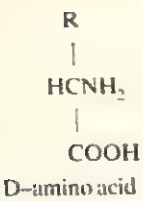
THE term 'protein' is derived from the Greek word 'proteios' which means 'primary or holding first place'. As the name indicates, this group of compounds is the most important of cell constituents. They are present in cytoplasm as well as the cell membrane of all cells without exception. Mammalian muscle contains about 20% protein, blood plasma 7%, cow's milk 3.5%, cereals 12%, beans, nuts and pulses about 20%. Besides forming structural elements of the body and important food constituents, they are also present in articles of daily use such as silk, wool and leather. A group of substances called enzymes which are agents responsible for all the chemical transformations taking place in the body are also protein in nature. Many of the hormones which regulate the chemical and other processes in the body are also proteins. Some of the disease producing substances called viruses and also the disease curing materials, antibiotics, are also proteins.

In addition to C, H and O which are present in carbohydrates and lipids, proteins also contain nitrogen. The nitrogen content is fairly constant and forms about 16% of the molecular weight of the protein. Small amounts of sulphur and phosphorus are also present in most proteins. Proteins are large molecules and can be split into smaller units by hydrolysis. These units are named 'amino acids', and are about twenty in number. By combining these twenty amino acids in different sequences and different numbers, an infinite number of proteins can be formed and in fact do occur in nature. It is analogous to the infinite number of words that can be formed with the 26 letters of the alphabet. While the words have to be restricted in length, there is no such restriction regarding the number of amino acids that may form a protein.

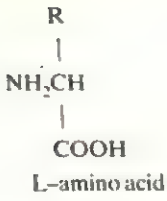
The bacterium, Escherichia coli, is estimated to contain about 5,000 different types of compounds which include some 3,000 different kinds of proteins and 1,000 nucleic acids. In the human organism, there may be 100,000 different kinds of proteins. None of the proteins of E. coli is identical with any of the human proteins. Thus, in about 1.5 million species of living organisms, there are probably 10^{10} to 10^{12} different kinds of protein molecules and about 10^{10} different kinds of nucleic acids.

AMINO ACIDS

With only a couple of exceptions, all amino acids have each one amino group and one carboxylic group. The amino group is attached to the alfa carbon. The general formula 'R CH NH_2COOH' will represent all amino acids, where R stands for H or other groups. The carbon holding the amino group is asymmetric as can be seen below:



D–amino acid



L–amino acid

All naturally occurring amino acids are of the L– configuration.

Classification of amino acids

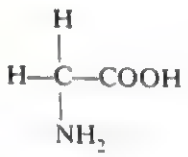
Amino acids can be classified into three groups depending on their reaction in solution: (1) neutral (2) acidic and (3) basic.

I. Neutral amino acids

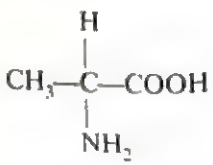
This comprises the largest group and can be further subdivided into aliphatic, aromatic, heterocyclic and sulphur containing amino acids. They have all of them one amino and one carboxylic group, each.

A. Aliphatic amino acids

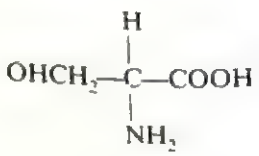
1. Glycine (Gly)
   (aminoacetic acid)



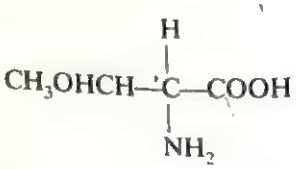
2. Alanine (Ala)
   (α - aminopropionic acid)



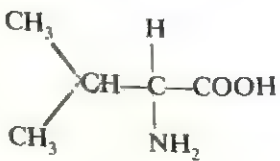
3. Serine (Ser)
   (α – amino, β - hydroxy propionic acid)



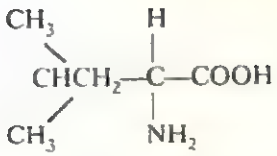
4. Threonine (Thr)
   (α - amino, β - hydroxy butyric acid)



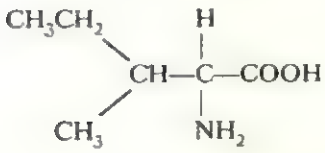
5. Valine (Val)
   (α - aminoisovaleric acid)



6. Leucine (Leu)
   (α - aminoisocaproic acid)

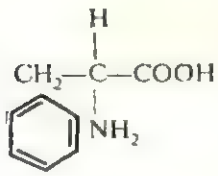


7. Isoleucine (Ile)
   (α - amino, β - methyl valeric acid)

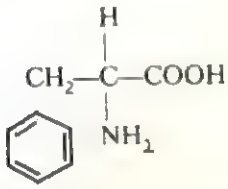


B. Aromatic amino acids

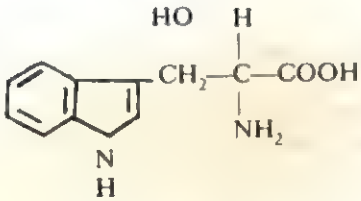
8. Phenylalanine (Phe)
   (α - amino, β phenyl propionic acid)



9. Tyrosine (Tyr)
   (p - hydroxyphenylalanine)

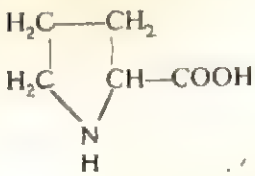


10. Tryptophan (Trp)
    (α - amino, β - indole propionic acid)



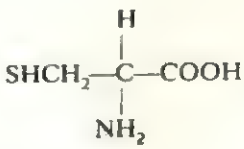
C. Heterocyclic amino acid

11. Proline (Pro)
    (Pyrrolidine - 2 - carboxylic acid)

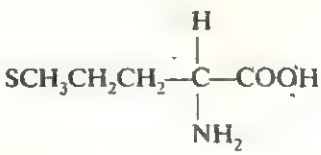


D. Sulfur containing amino acids

12. Cysteine (Cys)
    (α - amino, β - mercapto propionic acid)

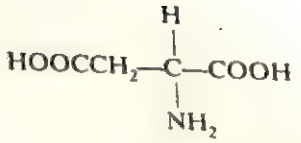


13. Methionine (Met)
    (α - amino,γ-methylthio - n - butyric acid)

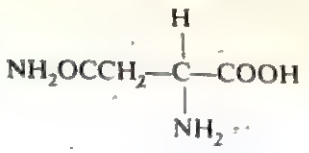


II. ACIDIC AMINO ACIDS

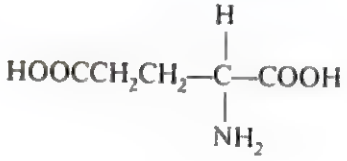
14. Aspartic acid (Asp)
(α - aminosuccinic acid)



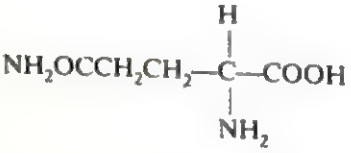
15. Asparagine (Asn)
(aspartic acid amide)



16. Glutamic acid (Glu)
(α - aminoglutaric acid)

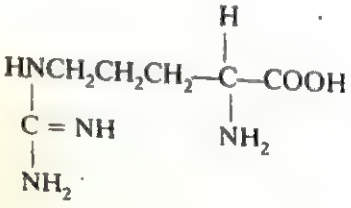


17. Glutamine (Gln)
(glutamic acid amide)

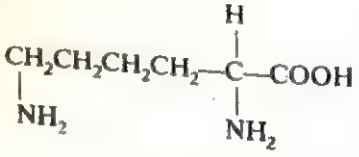


III. BASIC AMINO ACIDS

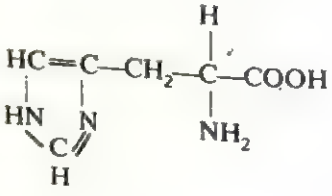
18. Arginine (Arg)
(α - amino, δ - guanidino
- n - valeric acid)



19. Lysine (Lys)
(α,ε-diaminocaproic acid)



20. Histidine (His)
(α - amino, β - imidazole propionic acid)



Note:

1. Glycine, the neutral, aliphatic amino acid, is optically inactive since it does not have an asymmetric carbon.
2. Proline, in the heterocyclic group, does not have a free amino group, but only a basic pyrrolidine ring. It cannot be represented by the general formula R $CHNH_2COOH$.
3. The acidic amino acids — aspartic and glutamic — have each one amino and two carboxylic groups per molecule (monoaminodicarboxylic). Their amides — asparagine and glutamine — are, however, neutral on account of the additional amide group.
4. The basic amino acids — arginine and lysine — have each two basic groups (guanidine and amino group in case of arginine and two amino groups in case of lysine) and one carboxylic group. Histidine has a basic imidazole ring in addition to the amino group.

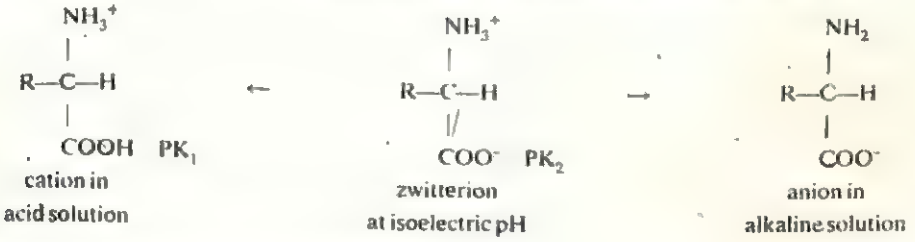
Occurrence: All the 20 amino acids listed above occur in almost all proteins. The acidic amino acids aspartic and glutamic are particularly rich in cereal proteins; the basic amino acids lysine and hydroxylysine occur in large amounts in the proteins of the connective tissue e.g. collagen. A few other amino acids or their derivatives occur in tissues as intermediate products during metabolic processes and will be considered at the appropriate time.

Properties of amino acids

Physical properties: They are colorless, crystalline substances generally soluble in water, slightly soluble in alcohol and insoluble in ether. Tyrosine is soluble in hot water only while cystine is insoluble even in hot water. They decompose on heating. All naturally occurring amino acids are levo rotatory except glycine which is optically inactive.

Formation of Zwitterions: On account of the presence of both acidic and basic groups which are readily ionizable, the amino acids behave as 'amphoteric electrolytes' and give both anions and cations in solution (zwitterions).

The dissociation of a monoamino-monocarboxylic acid can occur in stages thus —



They can combine with either acids or bases.

Titration of amino acids:

The amino acids can be titrated with either acids or bases. Each amino acid has at least two pK values on account of the amino and carboxylic groups. Some have more on account of the additional basic and acidic groups present in their molecules. At a pH which is usually the mean of all the pK vlaues for that amino acid, the molecule carries equal amounts of

positive and negative charges and is hence electrically neutral. If an electric current is passed through a solution of the amino acid at that pH, its molecules do not move either to the anode or cathode. The pH at which it gives off equal number of anions and cations and does not show any migration when subjected to an electric field is referred to as the 'isoelectric pH' of that amino acid. Above the isoelectric pH, it ionizes to give more of negative ions and migrates towards the anode. The reverse happens below the isoelectric pH. The amino acid ionizes with a net positive charge and migrates towards the cathode.

The isoelectric pHs of a few amino acids are presented in Table 4-1.

TABLE 4-1

Isoelectric pHs of some amino acids:

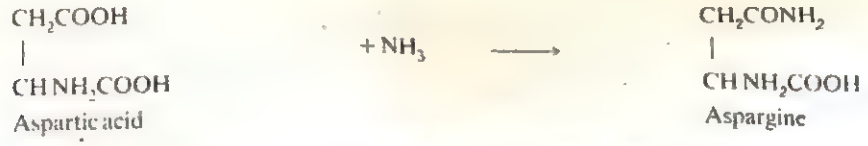
Aspartic acid	...	2.77	Glutamic acid	...	3.22
Phenyl alanine	...	5.48	Tyrosine	...	5.66
Tryptophan	...	5.89	Alanine	...	6.00
Proline	...	6.30	Histidine	...	7.59
Lysine	...	9.74	Arginine	...	10.76

Some amino acids like tryptophan, tyrosine and phenylalanine absorb ultraviolet rays at 260-290 mu. This property enables the identification of not only these amino acids but also the proteins which contain them.

Chemical properties. *Due to the –COOH group:*

(i) They can form esters with alcohols and salts with bases.

(ii) With ammonia, they form the corresponding amides. The amides of aspartic and glutamic acids — aspargine and glutamine — are of importance in the transport of ammonia in the body.



(iii) Lithium borohydride reduces the C-terminal amino acid to the corresponding α - amino alcohol.

(iv) Hydrazine (NH_2NH_2) will cleave all peptide bonds and convert all the amino acids except the C-terminal amino acid to the corresponding hydrazides.

Due to the —NH_2 group

(i) They can form salts with acids.

(ii) The amino group can be methylated or benzylated.

(iii) Nitrous acid will react with the amino group to liberate nitrogen and form the corresponding hydroxy acid.

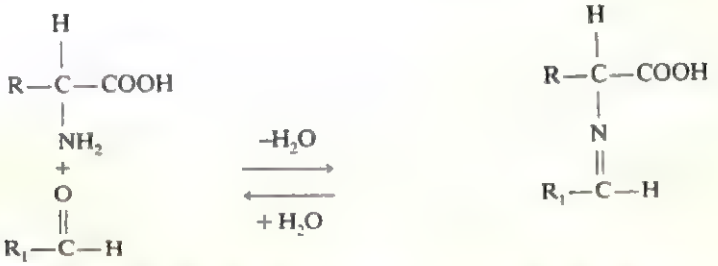
$$R\,CH\,NH_2COOH + OHNO \rightarrow R\,CHOH\,COOH + N_2 + H_2O$$

The nitrogen can be collected and measured. This is one of the methods for the estimation of amino acids.

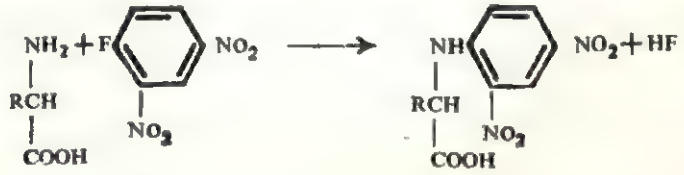
(iv) Formaldehyde reacts with the $-NH_2$ group to form a methylene compound.

With the amino group thus rendered non-ionizable, the amino acid can be readily titrated with standard alkali and estimated. (When the amino group is also allowed to ionize, it interferes with the titration by forming zwitterions and a sharp end point is not reached). 'Formol titration' is another method for the estimation of amino acid (Sorenson).

Amino acids react reversibly with other aldehydes to form additive compounds called Schiff's bases:

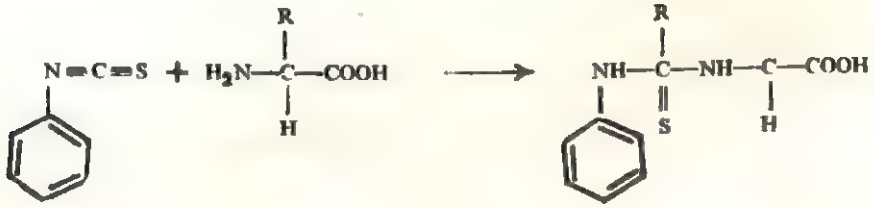


(v) Reaction with Sanger's reagent: 1-fluoro-2, 4-dinitrobenzene (FDNB), called Sanger's reagent, condenses with the free amino group in an alkaline medium.



The reaction can take place even with the amino acid in the polypeptide structure of protein at what is known as the N-terminal of the polypeptide. The compound so formed can be isolated after protein hydrolysis and identified.

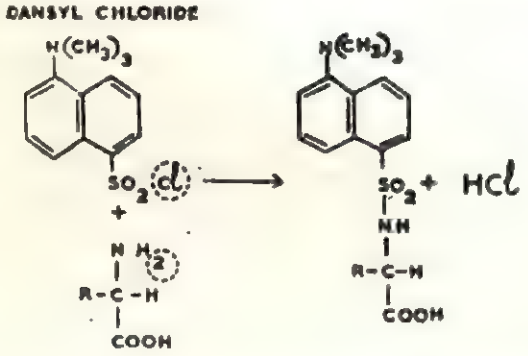
(vi) Edmann reaction: A similar reaction with phenyl isothiocyanate also enables the identification of the N-terminal amino acid.



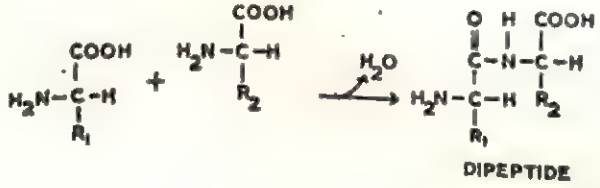
(vii) Ninhydrin reaction: When amino acids are heated with ninhydrin, the amino group is removed and a condensation product is formed with ninhydrin which has a bright blue colour. This can be used for colorimetric estimation of amino acids and in staining chromatographic strips.

Proline and hydroxyproline give a yellow color in ninhydrin reaction.

(viii) The N-terminal amino acid also combines with 1-dimethylaminonapthalene-5-sulfonyl chloride (dansyl chloride) to form a fluorescent dansyl derivative.

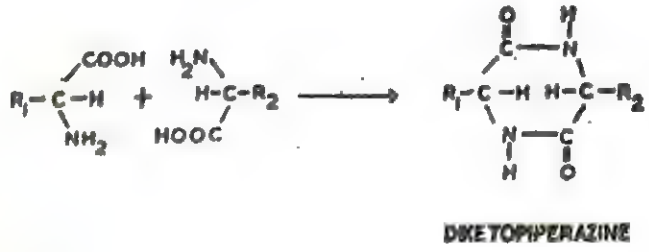


(ix) The peptide: Two amino acids interact by the amino group of one of them combining with the carboxylic group of the other through what is known as the 'peptide' bond (–CONH–).



The compound formed is a dipeptide. Hofmeister and Fischer have independently proposed (1902) that the peptide bond is the main type of linkage between amino acids in the protein structure.

(x) The amino acids may also interact by condensation between both the amino groups and both the carboxylic groups to form a ring compound called diketopiperazine.



Synthesis of Peptides: This can be brought about by first activating the COOH of an amino acid by converting it to its acid chloride or anhydride or thioester and then to make it react with the amino group of a second amino acid.

$$RCHNH_2\,COCL + NH_2\,CHR^1COOH \longrightarrow \underset{\text{(dipeptide)}}{RCHNH_2\,CONHCHR^1\,COOH}$$

By repeating the process, long polypeptide chains can be synthesized. But it is a tedious and time consuming process.

Polypeptide chains with 16 amino acid residues were synthesized early in the century by Emil Fischer. But, it was only in 1953 that the first naturally occurring polypeptide — oxytocin — was synthesized by du Vigneaud and his colleagues.

Peptides of physiological importance:

Glutathione: This is a tripeptide containing glutamic acid, cysteine and glycine. It is present in the erythrocyte and several tissues and helps in maintaining optimal redox potential in the cell and in keeping the enzymes in an active state by preventing the oxidation of their –SH (sulfhydril) groups to –S–S– (disulfide) groups.

Bradykinin and Kallidin: These are small polypeptides containing 9 and 10 amino acids respectively. They are formed by partial hydrolysis of plasma proteins by snake venom. They are powerful vaso-depressors and inhibitors of the heart.

Tyrocidin and Gramicidin: They are cyclic peptides containing ten amino acids each. The polypeptide chain forms a ring structure by the terminal two amino acids also combining with each other by peptide linkage. They are useful as antibiotics.

Posterior pituitary hormones oxytocin and vasopressin, hypothalamic hormones like somatostatin, intestinal hormone gastrin, hypertensins from the kidney and the enkephalins from brain are also small peptides of profound biological importance and will be dealt with in the appropriate chapters.

Purification and Characterization of Proteins:

Proteins can be separated from a mixture by using some of their known physical properties. Non-protein organic and inorganic solutes are easily removed by dialysis using a semipermeable membrane.

A. Molecular size: Since proteins have different molecular sizes, this can form a basis for their separation.

1. **Density-gradient or zonal centrifugation:** A continuously varying density gradient of a sucrose solution is prepared in a plastic centrifuge tube. The concentration of sucrose is 20% in top layers and 60% in the bottom layers. The protein mixture is layered on top and centrifuged in a horizontal centrifuge. Individual proteins will separate out into bands, the lowest being the heaviest molecule.

After hydrolysis with an enzyme, the number of peptides formed can be separated by two dimensional chromatography on paper. Each protein has a characteristic pattern in the chromatogram and serves like a finger print in identifying the protein *(finger print technique)*.

3. The N-terminal amino acid is determined in each polypeptide chain by Sanger's reaction, Edmann reaction or by the dansyl chloride method. The C-terminal amino acid is determined by the lithium hydrobromide method or by hydrazinolysis. The free amino acids are determined by ion-exchange chromatography. Nowadays, autoanalyzers are available.

4. The original polypeptide chain is hydrolyzed into small peptides using a differnet hydrolytic agent from the one used in step 2, so that the polypeptide chain is now broken at points different from the earlier hydrolysis. (If peptic digestion is used in step 2, tryptic digestion may be used in this step).

5. The amino acid composition of the small peptides formed now is again determined by repetition of step 3.

6. By comparing the amino acid sequences of the peptides in the two series obtained by hydrolysis at different points and by suitably overlapping them, the exact amino acid sequence of the polypeptide chain can be determined. Thus, if the amino acid sequence in step 5 for the peptides is – – – – ABC, EDXF, CDMN, and BZYC and the sequence in step 3 is – – – ABCC, DMNED, XFB, and ZYC, and if A is the N-terminal and C the C-terminal amino acid of the polypeptide, then the amino acid sequence of the polypeptide has to be – ABCCDMNEDXFBZYC.

Using such techniques and after years of painstaking research, Sanger and Smith succeeded in elucidating the complete amino acid sequence and structure of insulin.

7. Another indirect method for the determination of the amino acid sequence is now available. The base sequence of DNA (gene) or the m-RNA concerned with the synthesis of the polypeptide chain can be determined. It can be interpreted, using the genetic code, to arrive at the amino acid sequence (see further under "Protein biosynthesis").

CHROMATOGRAPHY

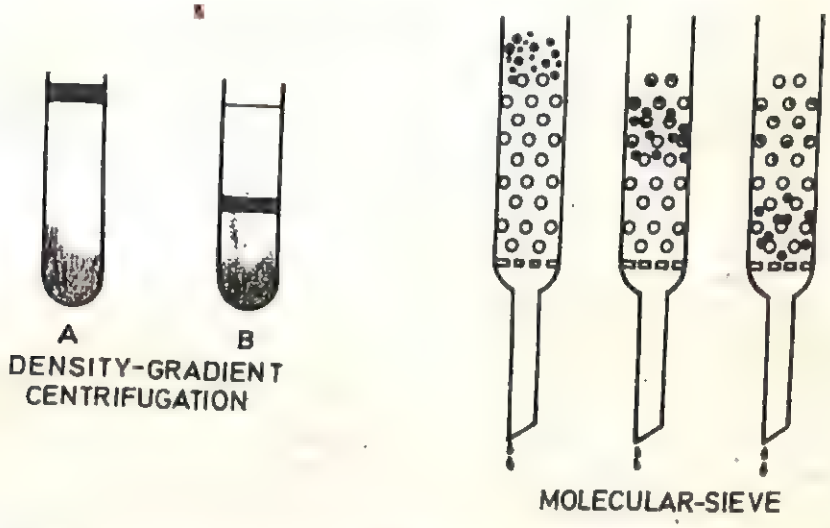
The term *'chromatography'* was first proposed by M. Tswett. He isolated plant leaf pigments on alumina column using nonpolar solvents like hexane. The technique known as chromatography has helped much in the separation and identification of amino acids from protein hydrolysates. Column chromatography and paper chromatography are the two commonly employed.

Column chromatography: A glass tube of suitable length is packed with a slurry made from a solid substance which has the property of adsorbing amino acids. When a solution of a mixture of amino acids is applied on to the top of this column and allowed to flow down through the column, different amino acids are adsorbed with different affinities on to the column. A suitable solvent is then allowed to flow through the column (a process described as 'elution'). Amino acids are extracted out, the ones having least affinity for adsorbent emerging out earliest. By collecting the solvent in small fractions from the continuously flowing eluate, (a 'fraction collector' which is an automatic device is used) the amino acids can be separated out as individual fractions, identified and estimated.

The material used for packing the column is of the nature of sulfonated polystyrene resin like Amberlite IR-120. It has a great affinity for cations and binds the basic amino acids strongly. Hence they are picked up last in the eluate. The acidic amino acids are eluted first followed by the neutral.

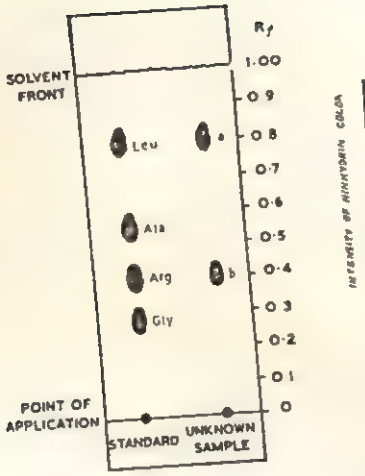
This is also called *"ion-exchange chromatography."*

Gel-filtration Chromatography or Molecular Sieving: Dextran (see under 'Polysaccharides – Chapter 2) granules wetted with water are packed in a column. The granules adsorb

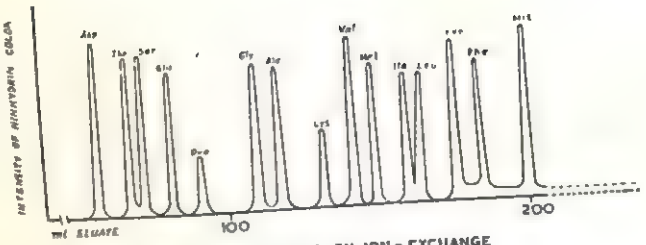


some water and form one phase while the excess water outside the granules forms the second phase. Small solute molecules can enter both phases whereas the larger solute molecules remain only in the external phase. If now a solvent is added on top of the column and eluted, the earliest portion of the eluate contains the large solute molecules to be followed later by the smaller molecules. Thus a separation is effected purely depending on the molecular size of the particles. Hence it is called molecular sieving or gel filtration. Sephadex, a cross-linked dextran, can be prepared with different degrees of cross-linkages and porosity to enable exclusion of various sizes of molecules. Thus, Sephadex G-25 excludes all but low molecular weight salts. It excludes even simple sugar molecules. Sephadex G-200, on the other hand, excludes substances with molecular weights between 5,000 to 200,000.

Paper chromatography: Instead of a column of a resin packed in a glass tube, a filter paper dipping into a mixture of two solvents, one of which is aqueous (hydrophilic) and the other an organic solvent (hydrophobic), is used. The organic solvents in common use are n-butyl alcohol, phenol, isobutyric acid and collidine. A minute quantity of the solution containing the mixture of amino acids is applied at a spot near the lower end of the filter paper. The system is kept in an airtight glass chamber and left alone for several hours. As the solvents slowly ascend along the filter paper (the advancing border of the solvent mixture is known as the 'solvent front'), the aqueous phase which has an affinity for the filter paper wets it



SEPARATION OF AMINO ACIDS BY PAPER CHROMATOGRAPHY



SEPARATION OF AMINO ACIDS BY ION-EXCHANGE CHROMATOGRAPHY

and forms what is known as the 'stationary phase'. This compares to the column of the column chromatography. The organic solvent which does not wet the paper forms the 'moving phase'. The amino acids (or in general any solutes that may be present in the mixture under analysis) get partitioned between the two phases depending upon their relative solubilities in the two phases. As the solvent front advances farther and farther the amino acids more soluble in the organic solvent travel farther upwards; while those which are more soluble in the aqueous phase remain closer to the point of application. If sufficient time is allowed, the different amino acids get separated out at different points along the length of the paper. The paper can be dried, stained suitably (say with ninhydrin) and the spots identified by running standard solutions of known amino acids. They can be estimated also by eluting the spots with suitable solvents followed by colorimetry or by densitometry on the paper itself.

RF Values: For a given set of experimental conditions, the ratio of the distance travelled by a particular amino acid from the point of application/the distance travelled by the solvent front is constant and is called the RF value. It is always less than one, since the distance travelled by the amino acid is always less than that travelled by the solvent front. By running a chromatogram under standard conditions, it is therefore possible to identify the amino acids by calculating the RF values.

Thin Layer Chromatography (T.L.C.): This is another technique, where, instead of paper, a suitable material like cellulose acetate, silica gel or other material is applied as a uniform thin film on a glass plate and used. The separation of the mixture is very much accelerated on this material.

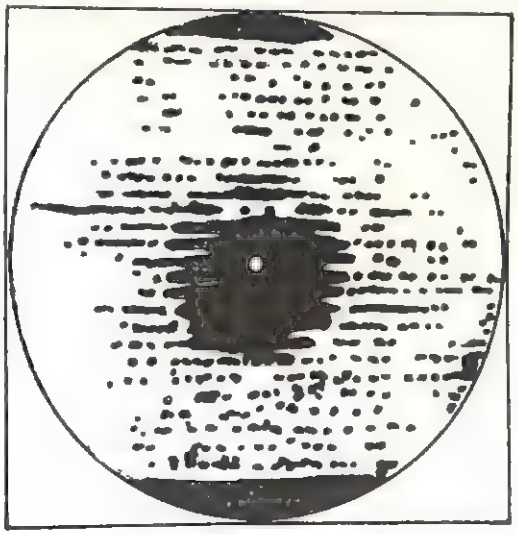
The amino acids in protein hydrolysates can be also analyzed by microbiological methods, spectrophotometry, isotope dilution methods and others the details of which are beyond the scope of this book.

Ion-exchange Chromatography: Diethylaminoethyl cellulose (DEAE-cellulose) contains positive charges at pH 7.0. It is an anion exchanger. Carboxymethyl cellulose (CM-cellulose) contains negative charges at pH 7.0 and acts as a cation-exchanger. The protein mixture is first run through a column containing one of them. The proteins get adsorbed on the cellulose. Then, buffers of different pH)in increasing order in case of CM-cellulose and decreasing order in case of DEAE-cellulose) are run through the column one following the other. Different proteins get eluted at different pHs. Selective adsorption onto inert materials like charcoal, alumina or silica gel and subsequent elution by buffers can also be used. If the substance used is such that it has affinity for only one protein (say an enzyme and its coenzyme or an enzyme and its substrate), then the method is called 'Affinity Chromatography'.

Another form of chromatography called "Gas-Liquid Chromatography", particularly useful in separation of fatty acids, was already mentioned in the chapter on 'Lipids'.

X-Ray Diffraction Studies on Protein Structure:

X-Rays, being electromagnetic waves of similar nature as light rays, can undergo diffraction. However, since the wavelength of X-rays is only about 1/1000 of that of light rays the spaces between the lines in an ordinary diffraction grating used for light rays will allow the X-rays to pass through without diffracting them. But the inter-atomic spaces in a crystalline substance will be small enough to diffract the X-rays and can act as a diffraction grating for them. A parallel beam of X-rays, after passing through a crystal of protein (or any other crystallizable substance) will get diffracted and scattered in different directions by the atoms in the crystal. Atoms with higher atomic numbers will diffract more than those with lesser atomic numbers. The pattern of the image produced can be photographed or detected by using ionization chambers such as Geiger counters. Each crystalline substance produces a highly characteristic image pattern depending on its molecular lattice i.e. the way the several atoms in its molecule are arranged about each other. The bond angles, inter-atomic distances etc., can be calculated. A simple example of the X-ray diffraction pattern of a crystal of myoglobin is shown below.



X-ray diffraction pattern of myoglobin

By the use of such devices, the complex structure of macromolecules like proteins and nucleic acids were worked out.

STRUCTURE OF THE PROTEIN MOLECULE

Organization of the protein molecule

Primary Structure: The sequence of amino acids in a polypeptide chain is the primary structure of the protein (see Fig. 4-1). It is this very sequence which determines the further levels of organization of the protein molecule. In representing the primary structure, the N-terminal amino acid (the amino acid with the free amino group) is always written on the left end of the polypeptide chain and the C-terminal amino acid (with the free carboxyl group) at the right end of the chain.

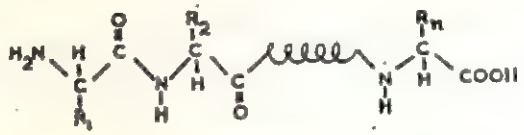


Fig. 4-1

Secondary Structure: Three types are possible: helical, reverse turn and pleated sheet. The tendency of the polypeptide chain is to arrange itself in space in such a way as to form a compact, tightly packed structure. If any crevices are present, they are usually packed with solvent molecules. By forming a coil, this purpose is served.

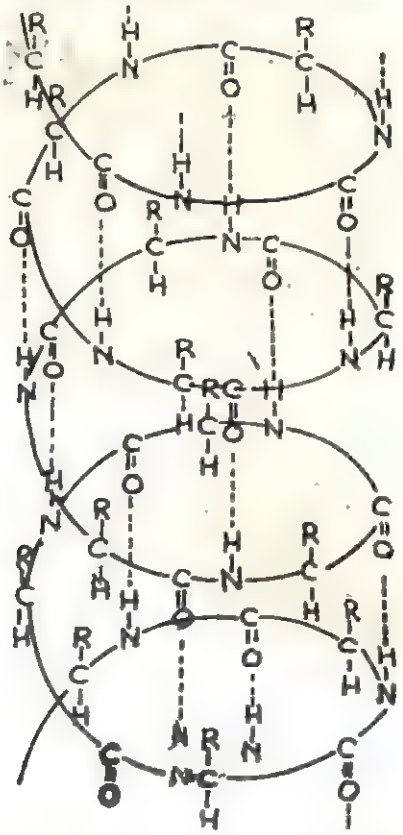


Fig. 4.2.a Alfa-helical Configuration of a Polypeptide chain.

The α -helix: (see Fig. 4-2.a). The polypeptide chain twists into a right handed screw. brining in the process the N-H group of one amino acid into close proximity of the C=O group of the fourth amino acid in the chain.

A hydrogen bond is formed between the C=O and N–H. In the coiled polypeptide chain, non-polar hydrophobic groups (side chains) tend to occupy the centre (interior) of the helix while the polar, nydrophilic groups (carboxylic, amino and hydroxy groups not participating in peptide bond formation) are oriented towards the periphery. Each turn of the helix contains 3.6 amino acid residues and is 5.4 Å long with a diameter of 6 Å.

Reverse Turns: The polypeptide chain may fold back on itself to change or even reverse the direction of the chain. Glycine and proline which do not have bulky side chains offer convenient spots in the polypeptide chain for folding to occur.

Pleated Sheet: A number of polypeptide chains lying side by side in an extended state (instead of coiled state) can form hydrogen bonds between C = O and N–H groups of neighbouring

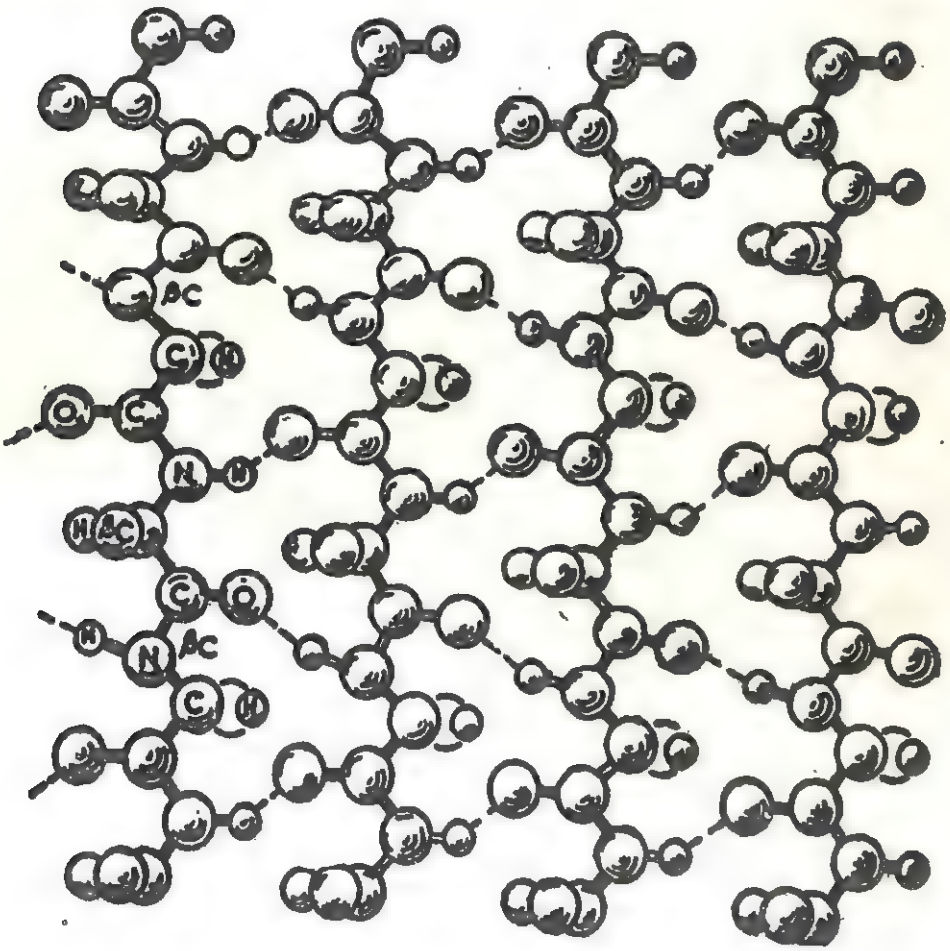


Fig. 4-2b Pleated Sheet Structure

polypeptide chains (in case of alfa helix, the bonds are formed within the same chain). The polypeptide chains may be parallel (if they run in the same direction) or anti-parallel (if the same chain takes a reverse turn and folds on itslef). Sheets of polypeptide chains are formed (see Fig 4-2 b).

A protein molecule may contain all three types of arrangement in different parts. Thus a part may form an alfa helix to be followed by a *domain* of pleated structure which may include some anti-parallel regions due to reverse turn of the polypeptide chain.

Tertiary Structure: The complex secondary structure will take on a three dimensional shape in which there is folding, looping and binding of the chain, including all its secondary features (the helix, the reverse turns and the pleated sheets) in such a way as to expose most polar groups to the surface and most non-polar groups to the interior. The final shape may be an ellipsoid, a globe or any irregular shape and is entirely determined by the intermolecular forces and bonds in the polypeptide chain. These forces are hydrogen bonds, ionic (electrostatic) bonds, hydrophobic interactions and other similar forces (see Fig. 4-3).

Hydrogen bonds: Mirsky and Pauling (1936) suggested that the major factor in maintaining the folded structure of a peptide chain is the hydrogen bond. Pauling and Corey postulated the pleated sheet structure of fibrous proteins. It is the hydrogen bonds within each helix and between neighbouring helices which maintain the structure.

The hydrogen of one -NH group can form a weak additional linkage with a neighbouring O or N. Most often, the hydrogen bond is formed between an -NH group and a -C = O group (see fig. 4-3, b.). Hydrogen bonds can also be formed between the -OH of tyrosine and a neighbouring free $-COO^-$ of aspartic or glutamic acids or N of histidine imidazole group.

Ionic bond (electrostatic bond or salt linkage): The non-peptide-forming -COO and NH_3^+ groups can form ionic bonds (see fig. 4-3, a.). Such bonds are stable only in the absence of water and are particularly strong in the hydrophobic portions of the protein molecule.

Hydrophobic interactions: The non-polar, alkyl side chains of amino acids, being hydrophobic, tend to come together (see fig. 4-3, c and d), just like small droplets of oil floating on water tend to come together to form larger droplets. The hydrophobic portions tend to form globular structures in which the non-polar groups tend to be in the interior and the polar groups on the surface of the molecule in contact with the aqueous solvent.

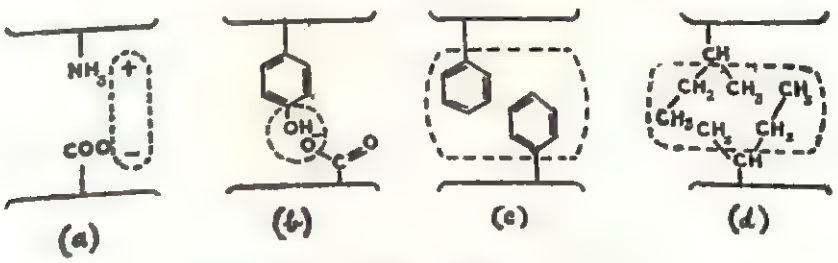
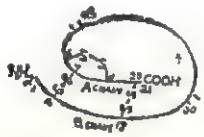


Fig. 4-3. Secondary linkages in the protein molecule.

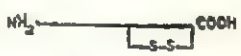
(a) Electrostatic bonds (salt linkages) (b) Hydrogen bond
(c) Hydrophobic bond (d) Hydrophobic interactions

Other forces: Electrostatic interactions between oppositely charged groups, interactions between dipoles and between dipoles and ions also help in maintaining the complex three dimensional structure of the protein molecule.

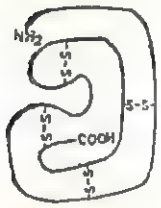
The tertiary structures of some proteins – proinsulin, oxytocin, ribonuclease and myoglobin – are shown schematically in Fig. 4-4.



i. **Proinsulin**



ii. **Oxytocin**



iii. **Ribonuclease**

iv. **Myoglobin**

Fig. 4–4.

Quaternary Structure: Some protein molecules are complexes containing more than one polypeptide chain. Each chain in the molecule has its own characteristic tertiary structure and is called a *subunit* or *monomer*. Two or more of them stay together by hydrophobic interactions, hydrogen bonds and electorstatic (salt like) bonds. The compound structure is called an *oligomer*. Hemoglobin is an example. It has four subunits – two alfa and two beta polypeptide chains, each having a tertiary structure similar to myoglobin (see Fig. 6-2 in the chapter on Blood and Body Fluids).

A detailed account of the structure of collagen and hemoglobin molecules and its relation to their function is given in the chapters 6 and 7.

(*v*) Upto the quaternary level of organization functional units of one protein only participate. A quintary level of organization is also described wherein groups of proteins exist in a cell as 'protein-protein aggregates'. They form insoluble particles in most cases. The enzymes of the citric acid cycle occur in the mitochondria in such a state. Similarly, the enzymes and coenzymes concerned with the conversion of pyruvic acid to acetyl coenzyme–A consisting of three separate enzymes and their coenzymes also occur in a quintary level of organization.The enzyme complex with acyl carrier protein which is concerned with the cytoplasmic synthesis of fatty acids is also an example of the quintary level of organization of protein molecules.

Physical and chemical properties of proteins:

A purified protein in said to be tasteless and odorless. Most proteins are soluble in water, but, on account of the large molecules, they form colloidal solutions.

(i) Molecular weights of proteins: They vary from a few thousands to several million The molecular weights of some common proteins are given in Table 4-3.

TABLE 4-3

Name of protein	*Mol. Wt.*	*Name of protein*	*Mol. Wt.*
Cytochrome–C	15,600	Myoglobin	17.000
Pepsin	35,500	Egg. albumin	44,000
Insulin	47,800	Hemoglobin	63,000
Serum albumin	69,000	Serum gamma globulin	176,000
Catalase	250,000	Fibrinogen	330,000
Myosin	850,000	Rabbit papilloma virus	47,000,000
Tobacco mosaic virus	59,000,000		

(ii) Amphoteric nature of protein: While one –COOH and one $-NH_2$ of each of the amino acids are involved in the peptide bond there are several free $-NH_2$ and other basic groups from basic amino acids and carboxylic groups from acidic amino acids which can ionize in solution producing both anions and cations. The protein molecule, like the amino acid molecule, thus exists as a zwitterion in solution. It can combine with both acids and bases and thus is an amphoteric substance.

For every protein in solution, there is a particular pH at which the number of anioms formed is exactly equal to the number of cations, and the solution is electrically neutral. That pH is called the 'isoelectric pH' of that protein. When a protein solution is maintained at a pH equal to its isoelectric pH, protein molecules do not migrate when subjected to an electric field. At pH values above or below the isoelectric pH, they carry a net negative or positive charge and migrate to the anode or cathode as the case be. This property of proteins finds practical application in the separation of proteins from a mixture (eg. plasma proteins) and is referred to as 'electrophoresis'. (For further details, the chapter on 'Blood and Body Fluids' may be referred).

(iii) Many proteins have been purified and obtained in a crystalline form.

(iv) Viscosity of protein solutions: Solutions of fibrous proteins are highly viscous (e.g. fibrinogen) compared to globular proteins like albumin.

(v) Hydration of protein: When brought into contact with water, protein molecules adsorb water and swell up. The polar groups like –COOH, $-NH_2$ and –OH become hydrated. Electrolytes, alcohols or sugars in high concentration will compete for the water of hydration, dehydrate the protein and precipitate it from solution.

(vi) Precipitation of proteins: Precipitation can be brought about by salting out or by addition of alcohol or acetone as mentioned above.

Precipitation is also brought about by salts of heavy metals like zinc, copper, mercury and lead. The precipitation is best in an alkaline medium, since the heavy metals interact with protein under those conditions.

Alkaloidal reagents like tannic acid, pircric acid, trichloracetic acid and ferrocyanide precipitate the protein when present as cation (protein$^+$); an acidic medium is optimal for this.

(vii) Heat coagulation of protein: Most proteins, when heated in solution, become insoluble and get precipitated in the form of a coagulum. Coagulation occurs best when the protein solution is kept at its isoelectric pH. During heat coagulation the protein molecule undergoes a change described as 'denaturation'.

(viii) Denaturation of proteins: Not only heat, but several other physical and chemical forces can bring about denaturation of a protein. Strong acids and alkalies, alcohol, acetone, concentrated solutions of urea and salicylates, exposure to ultra-violet rays or X-rays and even vigorous mechanical shaking can bring about denaturation. The cross linkages like the hydrogen bonds and disulfide linkages are broken and the protein molecule loses its characteristic secondary and higher levels of organization. There is an unfolding of the coiled structure of the polypeptide chain and a more or less fibrous type of protein is formed. The denatured protein is less soluble in water, its isoelectric pH is different from the original (or 'native') protein and it is biologically inactive in most cases.

(ix) Reversibility of Denaturation: F. White and C.B. Anfinsen studied the denaturation of ribonuclease by 8M urea in the presence of a reducing agent, β–mercaptoethanol. The ribonuclease molecule was completely unfolded due to a break of the four disulfide linkages. The enzyme activity was lost. But, when the urea and mercaptoethanol were removed by dialysis, the enzyme activity returned. This shows that the peptide chain has the inherent capacity to form the tertiary structure on its own. It was calculated that the chance of forming the disulfide linkages between the 8 cysteine residues in the correct positions was 1 in 105. The molecule was able to choose the correct position every time.

A nuclease from staphyllococcus cells becomes denatured and loses all biological acitvity on acidification to pH 3.0. The activity is regained at pH 7.0. This also indicates a restoration of the native conformation of the enzyme molecule. In this case, there are no disulfide linkages at all.

Renaturation may occur spontaneously in some cases in course of time. In such cases, the biological activity is also restored.

Color reactions of proteins:

Proteins give a number of color reactions with reagents on account of the amino acids contained in them.

Biuret test: It is so called because a substance called 'biuret', obtained by heating urea, gives a positive test. The characteristic linkage in biuret is the –CONH– linkage or the peptide linkage. When the protein solution is made alkaline with NaOH and a drop of dilute copper sulfate solution is added to it, a pink or violet color is obtained. It is due to the formation of a colored co-ordination complex between the cupric ions, the nitrogen of the –CONH– and the oxygen of water.

Millon reaction: Phenolic compounds, when heated with mercuric nitrate in nitric acid and traces of nitrous acid, develop a red color. The tyrosine present in the protein is responsible for the test.

Sakaguchi reaction: Guanidines (present in the arginine of protein) in alkaline solution give a red color with alfa naphthol and sodium hypochlorite.

Nitroprusside test: Cysteine gives a red color with sodium nitroprusside in dilute ammoniacal solution.

Aldehyde reaction for tryptophan: Indole derivatives give a purple color with p-dimethyl aminobenzaldehyde in sulfuric acid (Ehlrick reagent).

Folin's reaction: Amino acids give a red color with sodium 1,2-napthoquinone-4-sulfonate in alkaline solution.

Classification of proteins:

Proteins are classified into three groups: A Simple proteins, B.Conjugated proteins and C Derived proteins.

A. Simple proteins: On hydrolysis, they yield only amino acids.

(i) Albumins: They are soluble in water and are heat coagulable. They can be precipitated from solution by saturating the solution with ammonium sulphate. Egg albumin, serum albumin, lactalbumin from milk and soya bean albumin are some examples.

(ii) Globulins: They are insoluble in water, but soluble in dilute salt solutions. Like albumins, they are also heat coagulable. They are precipitated from solution by half saturation with ammonium sulphate. They occur togther with albumins in the same sources such as milk, egg and serum.

(iii) Glutelins: They are soluble in dilute acids and alkalies, but insoluble in neutral solvents. The proteins of wheat, rice and other cereals belong to this group.

(iv) Prolamines: They are soluble in 70% alcohol. They are rich in proline and occur in cereals like corn, barley and others.

(v) Scleroproteins (albuminoids): They are insoluble in all the solvents considered so far. They are animal proteins present in hair, horn, hoof, nails, cartilage and bone. Collagen of bone, keratin of hair and fibroin of silk are a few examples. They are, in general, rich in sulfur containing amino acids.

(vi) Histones: They are soluble in water, dilute acids and salt solutions. They have isoelectric pH on the alkaline side, because they contain large amounts of basic amino acids like arginine. The globin of hemoglobin and the protein moiety of several nucleo-proteins belong to this group. They can form salt-like compounds with acidic substances eg. nucleic acids.

(vii) Protamines: These are the simplest of the proteins with very low molecular weights. They are strongly basic and are rich in arginine. They occur in the nucleoproteins of the sperm.

Histones and protamines, since they normally occur in conjugated proteins like hemoglobin and nucleoproteins, may be also classed under derived proteins.

B. Conjugated proteins:

The proteins in this group are combined with a non-protein group called the 'prosthetic group'.

(i) Nucleoproteins: These are considered in detail in the next chapter.

(ii) Proteoglycans and glycoproteins: In hyaluronic acid, heparin and chondroitin sulfate (see Mucopolysaccharides in the chapter on 'Carbohydrates'), the repeating unit is a disaccharide containing glucosamine (or galactosamine) combined with a uronic acid. Proteins conjugated with such mucopolysaccharides are called "Proteoglycans".

In others, the polysaccharide is made up of a single repeating monosaccharide – any one of glucose, galactose, mannose, N–acetylglucosamine, N–acetylgalactosamine, fucose (6–dexygalactose), arabinose and xylose. Sialic acids (eg. N–acetylneuraminic acid) are present in some. Proteins conjugated with such polysaccharides are called "Glycoproteins".

The oligosaccharide side chains are covalently attached to the polypeptide backbone. When the protein component is removed, the oligosaccharides are called "Glocosaminoglycans".

Gastric and salivary mucins, immunoglobulins, human chorionic gonadotropins, erythrocyte and lymphocyte cell membrane proteins are examples of glycoproteins.

Deficiencies of enzymes concerned in the synthesis as well as degradation of the glycoproteins and proteoglycans are met with in nature and give rise to several diseases involving the connective tissues. A detailed description of these is beyond the scope of this book.

(iii) Chromoproteins: The prosthetic group is a colored compound. Hemoglobin, flavoprotein and visual purple are a few examples.

(iv) Phosphoproteins: Phosphoric acid is attached to the OH of serine or threonine. Casein of milk and vitellin of egg yolk belong to this group.

(v) Lipoproteins: Protein is combined with phospholipids mainly. Lipoproteins occur in milk, blood serum, egg yolk and others. They are also important constituents of the cell membranes.

(vi) Metalloproteins: Fe, Co, Mn, Zn, Cu and Mg are some of the metallic ions associated with proteins. The proteins are usually enzymes and require the metallic ions as activators.

C. Derived proteins

They are products of denaturation or of partial digestion of proteins.

(i) Proteans: These are denatured protenis; e.g. fibrin from fibrinogen; myosan from myosin.

(ii) Metaproteins: On treatment with acid or alkali, the corresponding acid or alkali metaprotein is formed.

(iii) Coagulated proteins: They are also denatured proteins.

All the above three groups are called the 'primary derived proteins'. Those produced as a result of partial digestion are called 'secondary derived proteins'. *(iv)* Proteoses; *(v)* Peptones and *(vi)* Peptides are formed in that order when a protein is hydrolyzed in a stepwise manner. The molecular size diminishes gradually, but the polypeptide structure and protein reactions are still given by the products.

References:

Block, R.J. and Bolling, D: The Amino Acid Composition of Proteins and Foods., 2nd. ed. Thomas, Springfield, Ill, 1951.
Edsall, T.T. and Wyman, J.: Biophysical Chemistry, vol. I, Acad. Pr., N.Y., 1958.
Florkin, M. and Stotz, E.H. (eds): Comprehensive Biochemistry, Vol. 7 (Proteins): Elsevier, Amsterdam, 1963.
Haurowitz, F.: The Chemistry and Function of Proteins. 2nd ed., Acad. Pr., N.Y., 1963.
Lennarz, W.J.: The Biochemistry of Glycoproteins and Proteoglycans. Plenum Press, 1980.
Meister, A.: Biochemistry of Amino Acids, 2nd. ed. vols. 1 and 2., Acad. Pr., N.Y., 1965.
Neurath, H.: The Proteins, 2nd ed. Vols. 1 and 2. Acad. Pr., N.Y., 1963, 1964.
Steiner, R.F.: The Chemical Foundations of Molecular Biology., Van Nostrand. Princeton, N.J., 1965.

5

NUCLEIC ACIDS AND NUCLEOPROTEINS

NUCLEOPROTEINS are conjugated proteins having nucleic acids as their prosthetic groups. The protein is usually basic in nature (e.g. protamines and histones). Nucleoprotein is present in every living cell as well as in viruses and bacteriophages and is the essential constituent of the genes. The genes, which are themselves constituents of chromosomes, are responsible for the transmission of hereditary characters from the parent to the offspring. Reproduction and cell division are the functions of the chromosomes. Abnormalities in the nucleoprotein structure of the genes give rise to mutation if the abnormality is major, and hereditary disease (inborn error) if minor. Evolution of the species is itself an example of several such genetic changes superimposed over millions of years.

Miescher (1871) first isolated nucleic acids from pus cells by digestion with dilute hydrochloric acid. He realized that they were present mainly in the cell nuclei and named the material 'nuclein'. He later isolated the protein component also from ripe salmon sperm. It was a basic protein which he called 'protamine'. It was Altmann who first used the term 'nucleic acid' in 1889 and discovered the existence of two different types of nucleic acids. Chargaff, Khorana, Watson and Crick and several others contributed to elucidate the detailed chemistry and structure of these fascinating substances. Like proteins, the nucleic acids also have primary, secondary, tertiary and quaternary structure.

Nucleic acids can be broadly classified into (1) deoxyribonucleic acid (DNA), present in nuclei and some viruses. Small amounts are also present in the mitochondria; (2) ribonucleic acid (RNA), mainly present in the cytoplasm and some viruses. Since DNA is the main constituent of the chromosomes, the DNA content of tissues is fairly constant for each type of tissue. Since germ cells (spermatozoa and ovum) contain only half the number of chromosomes, the DNA content of these cells is only about half that of the other cells.

RNA is more general in distribution and occurs in cytoplasm as well as in the nucleolus. Ribosomes are the richest in RNA. Next come the mitochondria.

Isolation of Nucleic Acids: Nucleoproteins are first extracted from minced tissue with I-molar (I-M) NaCl solution. The protein part is then separated by saturating the extract with NaCl or by mild acid or alkali hydrolysis or better still by hydrolysis with proteolytic enzymes like trypsin. The protein gets precipitated by saturation of the solution with NaCl in the former case and can be removed. The nucleic acids can finally be precipitated by alcohol.

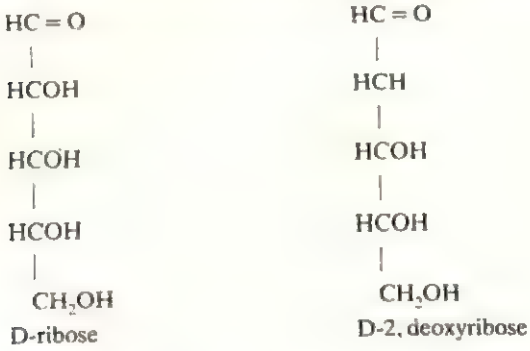
From the mixture of RNA and DNA, RNA can be removed by extraction with phenol in which it is soluble. Differential centrifugation, ion-exchange chromatography and other techniques are also available.

Characterization of D.N.A. and R.N.A. by Zone Centrifugation through Density Gradient:

An aqueous solution with a concentration gradient is set up in a centrifuge tube, so that the density of the solution increases linearly from top to bottom of the tube (say 5% sucrose solution on top to 25% sucrose solution at the bottom) (See P. 71). The nucleic acid solution is layered on top of the gradient and centrifuged at high speed for several hours. Molecules sediment through the gradient at a rate governed by the shape, size and molecular weight. At the end, when equilibrium is attained, the nucleic acids are separated out into discreet layers in the tube and can be collected dropwise by introducing a needle at the bottom of the tube. Thus R.N.A. molecules of 4S, 18S, 28S etc., can be separated out. (S-Svedberg unit) (see under ultracentrifugation).

Both DNA and RNA can be considered to be polymers of a simple unit called a 'nucleotide'. Each nucleotide is made up of a base, a sugar and phosphoric acid. If the phosphate is removed by hydrolysis, the residue consisting of a base and sugar is called a 'nucleoside'. The nucleoside can be further hydrolyzed to separate the base from sugar.

Sugar: The sugar moiety is D-ribose in RNA and D-2, deoxyribose in DNA.



They exist in the ring form as furanosides.

Base: Two types of bases are present — pyrimidine and purine.

Pyrimidine bases are derivatives of the pyrimidine ring. They are cytosine, uracil and thymine. Purine bases are derivatives having the purine ring. Adenine and guanine are the purine bases which occur in either of the two nucleic acids. The parent rings and the derivatives are shown in Fig. 5-1. (Please note that the numbering of the positions in the pyrimidine ring is different from that followed for the purine ring. This is in accordance with the latest international system).

Cytosine is 2-oxy-4-aminopyrimidine.
Uracil is 2, 4-dioxypyrimidine.
Thymine is 2, 4-dioxy-5-methylpyrimidine.
Adenine is 6-aminopurine.
Guanine is 2-amino-6-oxypurine.

They can all exist in the lactim form (oxygen is in keto form as shown in the structures in Fig. 5-1) or in the lactam form, in which the oxygen is present as –OH.

Cytosine and uracil are present in RNA while cytosine and thymine occur in DNA.

Beside the above six bases, several of their derivatives are also present in small amounts in some of the nucleic acids.

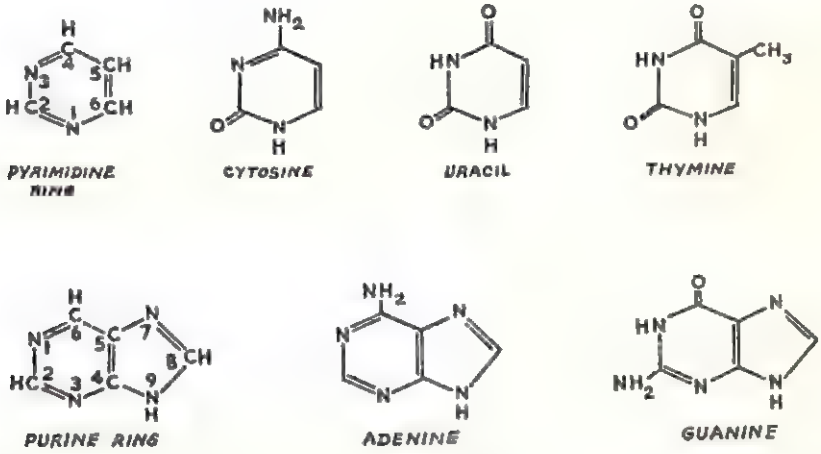


Fig. 5-1

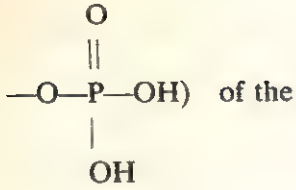
The base combines with ribose or deoxyribose by a C-N linkage in which the nitrogen at position 1 in case of pyrimidine base and at position 9 in case of the purine base and the first carbon of the sugar participate. The nucleoside thus formed combines with phosphoric acid by esterification of one of the alcoholic groups of ribose to form a nucleotide.

Uracil	+	sugar	⟶	uridine	+	H_3PO_4	⟶ uridylic acid
Thymine	+	"	⟶	thymidine	+	"	⟶ thymidilic acid
Cytosine	+	"	⟶	cytidine	+	"	⟶ cytidilic acid
Adenine	+	"	⟶	adenosine	+	"	⟶ adenylic acid
Guanine	+	"	⟶	guanosine	+	"	⟶ guanylic acid

Pseudouridine: In uridine, ribose is attached to N_1 of uracil by a C-N bond. But in psuedouridine, which occurs particularly in t-RNA, ribose is attached to C-5 of uracil by a C-C bond.

Structure of nucleic acids

A polynucleotide is formed by union of several nucleotides, each successive nucleotide being united to its immediately preceding one by forming a diester with phosphate. Only one of the hydroxy groups of ribose is esterified in nucleotide formation leaving two more hydroxy groups in case of ribose and one more hydroxy group in case of deoxyribose free for esterification with the phosphate (which itself has got a free enolic group —



—O—P—OH) of the neighbouring nucleoside. The Fig. 5-2 below illustrates the 3'—5', phosphate diester linkages of DNA.

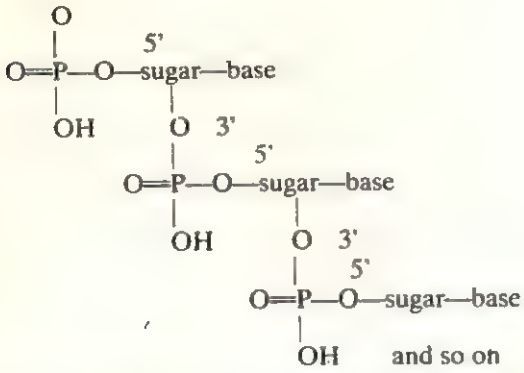


Fig. 5-2

A simple notation is used to represent the nucleotide sequence and linkage in a nucleic acid.

Adenosine-3'-phosphate is represented as 'Ap'. Adenosine-5'-phosphate as '$_p$A'.

If the molecule is DNA, the nucleotide series is prefixed with a 'd'.

$A_pG_pC_pU_p$ ... thus stands for a RNA molecule of 'adenosine-3' phosphate-guanosine-3'-phosphate-cytidine-3'-phosphate-uridine-3'-phosphate — ... etc.!

$dA_pT_pG_pC_p$ ... stands for a DNA molecule with the structure 'adenosine-3' phosphate thymidine-3'phosphate-guanosine-3'phosphate-cytidine-3'-phosphate ... etc'. Polynucleotide chains of enormous length can thus be formed just like polypeptide chains.

DNA: The purine bases adenine and guanine and the pyrimidine bases cytosine, methyl cytosine, hydroxymethyl cytosine, and thymine are present. The DNA content of a cell is said to be 10^9 nucleotide pairs, while that of a single gene is about 74 nucleotide pairs on the average. Adenine, guanine, thymine and cytosine derivatives all occur in about equimolar quantities.

The molecular weight varies from 6 to 100 million indicating presence of over 20,000 nucleotides per molecule. The D.N.A. of all the cells in the adult human body, if stretched out and arranged end to end, will have a length of 10^{10} miles. This is more than the distance from the earth to the Sun (9×10^7 miles).

The large polynucleotide structure of DNA is said to be organized in the form of a double helix (Watson and Crick, 1953), two polynucleotide chains coiling around each other in opposite directions. They are antiparallel. If, in one chain, the linkages are 3'-5', in the other it will be 5'-3'. Both are right handed helices, each coiled around the same axis. The two chains are held together by binding forces which include hydrogen bonds between the bases

of the two chains — i. adenine of one chain with thymine of the other by two hydrogen bonds and ii. guanine of one with cytosine of the other by three hydrogen bonds. The chains are thus complementary to each other (see Fig. 5-3).

If the base sequence in one is —A—T—C—G—A—T—C—G— etc.
that in the other will be —T—A—G—C—T—A—G—C etc.

The planes of the adjacent pairs are 3.4 Å apart. Each turn of the helix has a length of 34 Å (known as the *pitch of the helix*) and includes ten base pairs.

The double helical structure also forms alternating major and minor grooves along its long axis. The proteins of the nucleoprotein interact with the DNA molecules in these grooves. In bacteria and viruses, the two ends of the DNA molecules (the 3' and the 5' terminals) may be joined together to form circular DNA molecules.

The protein component of chromatin is made up of a nearly equal mass of small basic proteins, mainly histones. Non-histone, acidic proteins are also present in small amounts. The protein components form dense spherical particles called "nucleosomes", 12.5 nm in diameter, connected together by DNA filaments. The chromatin fibre is formed by a coiling of the nucleosomes along with the DNA filament. The diameter of the chromatin fibre is about 30 nm.

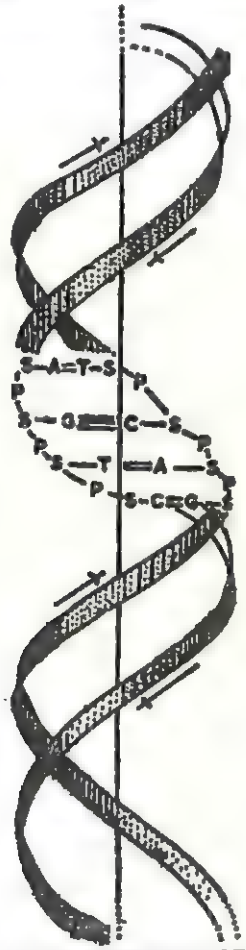


Fig. 5-3 Double Helix of DNA

RNA: Depending on the site of occurrence and functions, four types are described-messenger RNA (mRNA), soluble RNA (sRNA), ribosomal RNA and viral RNA. Like DNA,these also are polynucleotides linked together by phosphate diester bonds between 3' and 5' position of ribose moieties. The purine bases, adenine and guanine, and the pyrimidine bases, cytosine and uracil, are present.

But they are not present in equimolar amounts. The molecule is less organized than the DNA molecule and with a few exceptions occurs as a single strand. There is internal hydrogen bonding within the chain to keep it in a coiled position. A helical pattern is formed not between two strands, but by the same coiled strand folding back on itself (Fig. 5-4).

The purine and pyrimidine bases of the two strands lie in the plane which is perpendicular to the long axis of the helix, occupying the central core of the helix. The phosphate groups are located at the periphery. In addition to the hydrogen bonds between adjacent bases of the two strands, other forces also operate to hold the strands together.

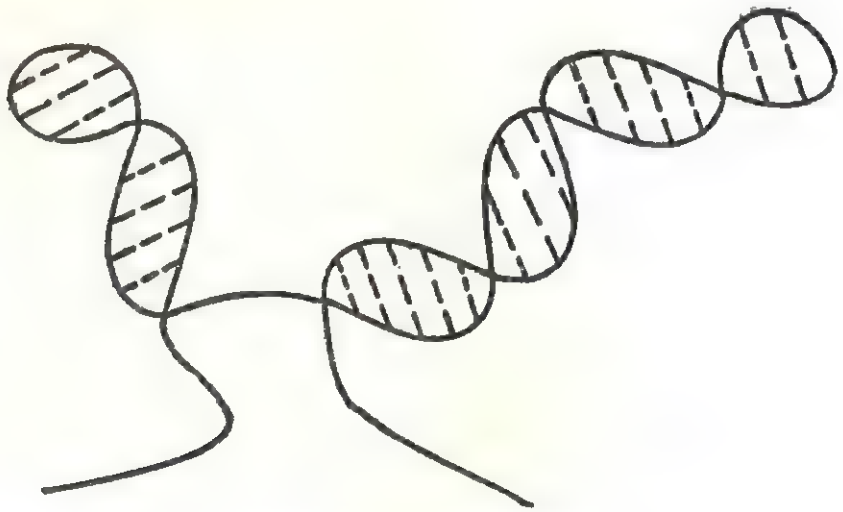


Fig. 5-4 Single stranded RNA molecule

The close packing of the bases of the two strands in the core causes an overlap of the electron clouds between the transient dipoles of uncharged atoms of the bases and helps to hold them together by Van der Walls forces. Further, the bases which have normally a tendency to associate themselves with water molecules are prevented from doing so when the bases are closely stacked over one another in the helix. This hydrophobic interaction of the bases also helps to keep them together.

The base pairing in the helical regions of RNA is similar to that in DNA i.e., A-U (instead of A-T in DNA) and G-C.

The molecular weight varies from 20,000 to 30,000 for soluble RNA to 2 million or more for ribosomal RNA and even more for viral RNA. The detailed nature of each of the types of RNA will be considered in the context of their function under 'SYNTHESIS OF PROTEIN' at a later stage.

Properties of DNA: On account of the purine and pyrimidine bases, nucleic acids absorb ultraviolet radiation strongly with an absorption peak at 260 mu.

The highly organized structure also makes it rotate polarized light to the right (dextro-rotatory.)

Solutions of DNA are highly viscous on account of the elongated and rigid molecules.

Since DNA has primary, secondary and tertiary structure like protein, it can also undergo denturation when heated or treated with strong acids or alkalies. The base pairs separate, causing a collapse in the helical structure and separation of the two strands. The denaturation process in this case is also called "melting" on the analogy of crystals melting and losing their structural characteristics. At a critical temperature termed the 'melting point' there is a sudden fall in the U.V. absorption peak and the optical rotation.

If the heat denatured DNA is slowly cooled, the denaturation is reversible, the helical structure is reformed and the two strands get together. This process is described as 'annealing'. By denaturing two different DNA molecules and annealing them later, it is possible to manipulate the structure to get a 'hybrid helix' consisting of one strand from each DNA.

Rapid cooling of the denatured DNA fixes it in a permanently denatured state and is called 'quenching'.

A solution of fuchsin in sulfurous acid (Fuelgen's reagent) gives a bluish violet color with DNA. It does not react with RNA. This is the basis of nuclear staining of histopathological tissue sections.

Nucleoproteins: The protein constitutent may be a simple polypeptide like protamine (as is sperm nucleoprotein) or a very large protein molecule of high molecular weight as in viral nucleoprotein. The protamines are basic in nature due to the presence of a large number of basic amino acids e.g., arginine, in their molecules. Their isoelectric pH is in the alkaline range and hence they exist as positively charged ions at physiological pH and readily combine (in a salt like manner) with the negatively charged nucleic acids. Histones are another group of proteins present in nucleoproteins. They are larger molecules, but they too have an isoelectric pH in the alkaline range.

Viruses: W.M. Stanley (1935) isolated the *tobacco mosaic virus.* Bawden and Pirie showed that it is a ribonucleoprotein. Both DNA containing and RNA containing viruses occur in nature. The viruses causing chicken-pox, mumps, rabies etc., are DNA viruses. Influenza, poliomyelitis and encephalitis are caused by RNA viruses. Viruses which infect bacteria are called "*bacteriophages*". They can be either DNA or RNA viruses.

Viruses are classified by some biologists as organisms. But they do not merit such classification, since they do not have any metabolism of their own.

The viruses can reproduce themselves inside living cells (host cells) but are incapable of doing so independently. This distinguishes them from bacteria. For the activity of the virus, its protein component is not necessary. Viral nucleic acid, isolated free of protein, can still proliferate itself in the host cell.

The molecular weights of viruses run into several million.

Some unusual bases like 5-methyl cytosine, 5-hydroxymethyl cytosine, 6-methyl aminopurine, 2-methyl adenine etc., occur in some DNA and RNA molecules, particularly associated with viruses, bacteriophage and some bacteria.

The nucleic acid is located at the centre of the 'core' of the virus. The protein moiety surrounds the core forming structures called 'capsomeres'. The entire structure may be further enclosed in an envelope (see Fig 5-5).

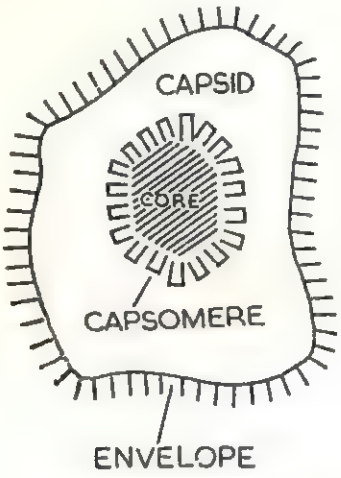


Fig. 5-5 Structure of virus

Nucleotides of biological importance

Apart from the nucleotides which form nucleic acids, there are several others present in nature or synthesized in the laboratory which have biologic importance.

Adenosine derivatives: Adenosine mono-, di-and tri-phosphates (AMP, ADP, ATP), cyclic AMP, S-adenosyl methionine (active methionine), adenosine 3'-phosphate-5'-phosphosulfate (active sulfate, PAPS), nicotinamide-adenine-dinucleotide and its phosphate (NAD and NADP), coenzyme-A, and 5-deoxyadenosyl derivative of cobalamine.

Guanosine derivatives: Gaunosine mono-, di- and triphosphate (GMP, GDP, and GTP) and cyclic GMP.

Hypoxanthine derivatives: Inosinc mono-, di- and triphosphates (IMP, IDP and ITP).

Uracil derivatives: Uracil mono-, di-and triphosphates (UMP, UDP and UTP), uridine diphosphoglucose (and uridine diphosphoglucuronic acid) (UDP-glucose and UDP-glucuronic acid)

Cytosine derivatives: Cytosine mono-, di-, and triphosphates (CMP, CDP, and CTP).

Synthetic derivatives: These do not occur in nature but are synthesized in the laboratory. Many of them are used as metabolic antagonists in clinical medicine in the treatment of infection and carcinomata and in preventing rejection of organ transplants.

5-Fluorouracil, 5-iodouracil, 6-thioguanine, 6-mercaptopurine, azauridine, azaguanine, allopurionl, cytarabine and vidarabine (nucleotides of cytosine and adenine where arabinose replaces ribose) are some examples. Some of these will be discussed further in the appropriate chapters.

References:

Chargaff, E.: Essays on Nucleic Acids. Elsevier, Amsterdam, 1963.
Davidsom, J.N.: The Biochemistry of the Nucleic Acids. 5th ed., Wiley. N.Y., 1965.
Florkin, M. and Stotz., E.H.: (eds): Comprehensive Biochemistry, vol. 8 part B, Elsevier, Amsterdam, 1963.
Khorana, H.F.: Some recent Developments in the Chemistry of Phosphate Esters of Biological Interest, Wiley, N.Y., 1961.
Michaelson, A.M.: The Chemistry of Nucleosides and Nucleotides, Acad, Pr., N.Y., 1963.
Preutz, M.F.: Proteins and Nucleic Acids, Elsevier, Amsterdam, 1962.
Steiner, R.F.Jr.: The Chemical Foundations of Molecular Biology, van Nostrand, Princeton, N.J., 1965.
Watson, J.: Molecular Biology of the Gene, Benzamin N.Y., 1965.
Watson, J. and Crick, F.: Nature, 171:964, 1953.

6

BLOOD AND BODY FLUIDS

THE evolution of the unicellular organism into the multicellular and highly complex vertebrate animal necessitated a fluid medium to bathe the myriads of cells and to circulate around them to supply to each cell nutrients and oxygen and to remove waste products including carbon dioxide. The blood and body fluids are hence evolved.

FUNCTIONS OF BLOOD

Blood can be considered to be a circulating tissue and serves very important functions to the other stationary tissues:

1. Transport of nutrients such as glucose, fatty acids and amino acids absorbed from gastrointestinal tract. They are distributed to all the tissues of the body.

2. Transport of waste products: Substances such as urea, uric acid, and creatinine are taken away from the tissues for ultimate excretion.

3. Gaseous exchange: Oxygen from lungs is carried to tissues and carbon-dioxide from tissues to lungs.

4. Transport of hormones: The endocrine glands secrete their hormones into blood and they are transported by blood to their target organs.

5. Maintenance of body pH: The efficient buffer systems present in blood enable it to transport the acid or alkaline metabolites from tissues and prevent their accumulation in tissues and thus help in maintaining a constant pH in and around the tissues.

6. Fluid balance: By virtue of colloidal osmotic pressure of plasma on one hand which draws in fluid and the capillary arterial pressure on the other which tends to force fluid out, a sort of a pumping mechanism is established which enables a constant exchange and flow of water between the blood and tissues via the lymph.

7. Maintenance of body temperature: Due to its constant circulation and the specific heat of water (which consitutes 55% of tissues and about 80% of blood) it maintains a uniform temperature in all tissues.

8. Defence against infection: By the phagocytic action of some of the leukocytes which engulf and destroy microorganisms and by the action of antibodies formed by the plasma proteins which neutralize the antigens, the blood provides a most important defence mechanism.

9. Prevention of hemorrhage: This is a mechanism of self-preservation. The blood which is normally fluid becomes solidified (clotted) at the site of an injury and thus prevents the loss of blood from the body.

The blood constitutes 6-8% of total body mass in adults. The blood volume is about 80 ml per kg body weight in men and 65 ml. in women. A 70 kg man will thus have 5.5 litres of blood.

The general features of blood are as follows:

Specific gravity ..	.. 1.060
Blood Cells:	40 - 45% by volume
Platelets..	200,000–400,000 per c.mm.
Leukocytes..	5,000–10,000 per c.mm.
Erythrocytes..	4,500,000–6,000,000 per c.mm.
Plasma:	50-60% by volume
Solids	8 - 9 gm/100 ml
Specific gravity	1.026
Osmotic pressure at 37°C	7.6 atmospheres
pH	7.33 - 7.51 (mean 7.4)

Erythrocytes:

The circulating erythrocytes and the total mass of erythropoietic cells from which they are derived are termed the 'ERYTHRON'. The erythrocytes are non-nucleated biconcave discs of diameter ranging from 6 - 9μ with an average of 7.5μ. The thickness is 1μ at the centre and a little over 2μ at the periphery. Hemoglobin is the main solid (31-33%) and is distributed in the cell within a stroma or meshwork composed of proteins and lipids.

The cytoskeleton of the erythrocyte is formed by three groups of proteins - *spectrin, actin* and *ankrin*. Spectrin is similar to myosin of muscle. An interaction similar to actin and myosin in muscle (see under the chapter on "The Cell and some Special Tissues") can occur between actin and spectrin in the erythrocytes. This interaction is facilitated by ankrin. On account of this cytoskeleton, the erythrocytes not only maintain their normal biconcave disc shape but can also suitably alter their shape while passing through narrow capillaries to enable free passage. *Glycophorin* is an important glycoprotein of the erythrocyte and is present mainly in the cell wall. It contains sialic acid and other carbohydrate moieties. Differences in its structure are responsible for the three main blood groups - A,B and O which contain the A,B and H or O antigens. The Lewis antigens Le^a and Le^b are not synthesized by the erythrocyte, but are added from the plasma onto the adult cells. They are present in the LDL and HDL (Low and High density lipoprotein) fractions of plasma as glycosphingolipids.

I, and P antigens are also glycoproteins. The M and N antigens are present in glycophorin itself. The M antigen containing protein is called glycophorin A and the N antigen containing one is called glycophorin B.

The erythrocyte cell membrane is readily permeable to (i) water, CO_2, urea, glucose (non-ionic molecules) (ii) HCO_3^-,Cl^- and OH^- (anions) and (iii)K^+ (cation). Only traces of sodium are present. (In plasma, mainly sodium is present and only small amounts of potassium). Similarly, magnesium is another cation present mainly in the cell while calcium is present mainly in the plasma.

The difference in the relative concentrations of Na^+ and K^+ between plasma and cell is mainfained by mechanisms involving active transport mediated by enzymes called ATPases.

Two such enzymes are identified - Na^+ - K^+ -dependent ATPase and Ca^{++} - dependent ATPase. These function as Na^+ and Ca^{++} pumps in removing these cations from the erythrocyte into the plasma.

LEUKOCYTES

Granular Leukocytes:

Polymorphs: They have a more active metabolism than erythrocytes. Glucose is completely metablolized to CO_2 and H_2O. They contain glycogen and also several more enzymes than erythrocytes. Of particular interest is 'alkaline phosphatase' content. It is genetically controlled. The neutrophil alkaline phosphatase (NAP) content is increased in Mongols and is virtually absent in chronic myeloid leukemia. Corticosteroid hormones increase NAP content. Polymorphs also contain small amounts of histamine. The cells have a short life. Usually they are destroyed by circulating toxins or by microorganisms in 6 to 8 hours. If not, they become senescent in 24 to 30 hours and die.

Eosinophil leukocytes: They contain histamine and plasminogen. They survive only for a few hours in circulation. They are attracted to sites of histamine or 5, OH-tryptamine production which they somehow render physiologically inactive.

Basophil leukocytes: They contain pepsin, 5, OH-tryptamine and relatively large amounts of histamine. They are also called 'mast' cells.

Lymphocytes: Two types can be distinguished, based on their life span. (1) Those which survive in circulation for only 2 or 3 days. (2) Those which survive for about 200 days. They are all indirectly produced by the thymus gland. In early fetal life, the lymphoid cells are 'seeded' from thymus in different parts of the body to become the lymphoid tissue of the adult. The thymus continues to exercise a humoral control over the lymphatic tissues even in the adult. The general metabolic pattern of the lymphocytes is similar to that of granular leukocytes. They are mainly concerned with the production of immunoglobulins.

The lymphocytes responsible for formation of immunoglobulins (circulating antibodies) are classed as, "B-lymphocytes". There are others which are responsible for cellular immunity, anti-tumour, anti-graft, anti-fungal etc. They are called the "T-lymphocytes'.

Monocytes: They originate probably in the bone marrow and spleen. They mainly function as macrophages (engulf foreign materials) and can enter various tissues as and when required to serve such functions. They take up antigens. Their association with the antigen, in some indirect and as yet ill-defined manner, facilitates the production of antibodies by the lymphocytes.

Platelets: They are the smallest formed elements circulating in blood. They are biconvex discs devoid of nuclei. The cell wall is rich in glycoprotein and mucopolysaccharides. They are rich in serotonin and a protein, which on release, can cause an increase in vascular permeability. This may play a role in inflammatory reactions. The platelets take part physically in hemostasis. The life span is about 7 to 14 days.

Hemolysis or Laking of Blood:

This is the term used to describe the process by which hemoglobin from the stroma of the erythroyte is released into the surrounding fluid (plasma or any other fluid in which the erythrocytes may be suspended). The cells maintain their shape and size when suspended in a sodium chloride solution of 0.9% concentration. This is isoosmotic (isotonic) with the fluid inside the cell.

If placed in hypotonic solution, water from the solution passes into the cells due to the osmotic gradient and causes them to swell and with a sufficiently low concentration of sodium chloride, the swelling of the cell and stretching of the cell membrane will be such that hemoglobin escapes through spaces produced in the stretched membrane. The release of hemoglobin may be also on account of the lowering of the concentrations of the other contents in the cell below a level optimal for binding the hemoglobin to the stroma. Normal erythrocytes commence to exhibit hemolysis in saline concentration of 0.45 to 0.39% and the process is complete (all hemoglobin comes out of the cell) at 0.33 to 0.3% saline concentration. A test performed to detect this range is known as the 'FRAGILITY' test.

Apart from salt concentration of the surrounding medium, certain substances like chloroform and ether dissolve away the lipids of the stroma and release the hemoglobin. Saponin, bile salts and detergents break down the lipid-protein complexes of the stroma and cause hemolysis. Toxic substances called hemolysins are present in snake venom and certain bacterial products which will also produce hemolysis of erythrocytes. Incompatibility of blood due to differences in blood groups will cause hemolysis in blood transfusions due to the presence of antigens.

In hypertonic saline, the red cells lose water and become shrunken and irregular (crenated in appearance).

Hemoglobin:

It is a conjugated protein containing 'HEME' as the prosthetic group. Heme containing proteins are characteristic of the aerobic organisms and are altogether absent in anerobic forms of life. Hemoglobin (R.B.C.), myoglobin (muscle), cytochromes, peroxidase and catalase (present in all cells) are all heme containing proteins.

Heme

It is an iron-porphyrin compound. The porphyrins are complex compounds with a tetrapyrrole structure, each pyrrole having the structure as shown in figure 6-1. Four such pyrroles are combined through – CH = (methylidyne) bridges to form a porphin.

The carbons in the four pyrrole rings (which are not linked with the methylidyne bridges) are numbered 1 to 8. The methylidyne bridges are referred to as α, β, γ and δ

The two hydrogen atoms in the -NH groups of pyrrole rings 2 and 4 may be replaced by ferrous iron (Fe^{++}) which will occupy the centre of the compound ring structure and establish linkages with all the four nitrogens of all of the pyrrole rings. If the iron were to be in the ferric state, it will carry a surplus + ve charge which is balanced by taking a OH^- from the medium. It may also be balanced by other anions such as Cl^- (the chloride compound is called 'hemin').

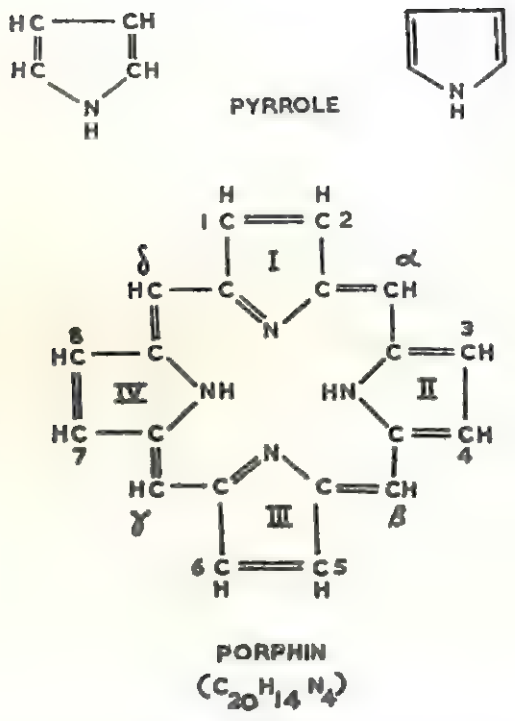


Fig. 6-1

In addition to containing ferrous iron in the centre, the hydrogens at positions 1 to 8 are substituted by different groups in different compounds. In the protoporphyrin which forms the parent compound of heme (protoporphyrin, Type III, series IX), the positions 1 to 8 are substituted by methyl ($-CH_3$) vinyl ($-CH = CH_2$); methyl, vinyl, methyl, propionic ($-CH_2$, CH_2, COOH), propionic and methyl groups in that order.

M	V	M	V	M	P	P	M
1	2	3	4	5	6	7	8

The porphyrin ring can be readily synthesized from simple substances (eg: glycine, succinate etc.) in the reticulo-endothelial cells.

Hemoglobin is formed by conjugation of heme with basic protein called globin. While the structure of heme is the same in hemoglobin from any animal source, the globin varies from species to species in its amino acid composition and structure.

Human hemoglobin has 0.34% iron and has a minimum molecular weight of 16,400 which represents a single iron and hence a single heme unit per molecule. But determinations using ultracentrifugation and other techniques indicate a molecular weight of 65,000 which means that four units (each containing one heme) form an aggregate molecule of hemoglobin. The aggregate can be broken in concentrated urea solution and other salt solutions into the four units of globin peptide chains, each with one heme. Mild acid hydrolysis removes the heme also leaving solitary globin polypeptide chains. The polypeptide can be broken into smaller peptides by enzymic hydrolysis and each such small peptide further analyzed to determine the amino acid sequence. By superimposing the information so gained, the amino acid sequence of the entire globin polypeptide can be determined.

By such analysis, 4 different amino acid chains are found to be present in the globin moieties from different sources. They are named the α, β, γ and δ chains. Human adult hemoglobin has two alfa and two beta chains. The alfa chain is made up of 141 amino acids with valine at the n-terminal and arginine at the c-terminal. The beta chain has 146 amino acids with valine at the n-terminal and histidine at the c-terminal. Portions of the peptide chains are helical while certain other portions are not. Each of the four polypeptide chains is arranged in the form of a tetrahedron with most of the polar and hydrophilic groups on the outer surface and the non-polar hydrophobic groups in the interior. This makes the protein readily soluble in water. In addition, each of the four polypeptide chains forms a pocket called the 'heme pocket'. The amino acids lining the pocket are strongly hydrophobic. A histidyl residue at position 87 of the alfa chain and position 92 of the beta chain are located in their respective heme pockets. The fifth co-ordination valence of the heme iron is linked to the nitrogen of histidine in these positions.

Histidine is also present in the 58th position of the alfa chain and the 63rd position of the beta chain, quite close to the heme pockets of the respective chains. A sixth coordination valence of the heme iron, which, in reduced hemoglobin is loosely combined to a molecule of water, forms, in the presence of oxygen, a bond with the nitrogen of this extra histidine through oxygen.

$$HbH_20 + 0_2 \leftrightarrow Hb0_2 + H_20$$

Thus iron in heme has six valencies as in ferrocyanide, $H_4\,Fe(CN)_6$.

On account of the hydrophobicity of the heme pocket, water is excluded and heme is thus placed in a medium of low dielectric constant. That is the reason why oxygen is able to complex with heme to form oxyhemoglobin without oxidizing heme iron to ferric form. If the dielectric constant were to be high, heme iron would have been oxidized to ferric form and a highly toxic superoxide ion $(O_2)^-$ would have been formed.

The propionic acid side chains of heme in positions 6 and 7 of the tetrapyrrole structure bind with lysine and arginine residues in the heme pocket. The alfa and the beta chains of the globin molecule are hence eminently designed to give attachment to four heme molecules and also to reversibly take up and part with oxygen by utilizing the sixth valence of heme iron. The globin molecule has also six –SH groups; two each in the alfa chain and one each in the beta chain.

A schematic representation of the hemoglobin molecule is shown in Fig. 6-2. The overall size of the molecule is 64Å × 55Å × 50Å.

Variations in Hemoglobin Structure

In the normal adult, most of the hemoglobin (90-95%) is the adult type (Hb A) with the structure described above with 2α and 2β chains in the globin molecule. A small amount (2-3%) of a variant HbA_2 is also present and is made up of 2α and $2\,\delta$ chains. In the fetus an altogether different hemoglobin called HbF (fetal hemoglobin) is present. It is made up of two α and two γ chains. This is gradually replaced by HbA. (At birth HbA is 85% and HbF is 15%). The fetal hemoglobin is resistant to denaturation by alkali whereas adult hemoglobin is readily denatured. This property is used in the identification of fetal hemoglobin.

Fig. 6-2 Myoglobin and Hemoglobin Molecules

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As many as 300 variants of human hemoglobin have been found by electrophoretic and chromatographic methods.

Variations in the structure of hemoglobin can cause alterations in its solubility, affinity for oxygen and ease of denaturation in vivo. There appear to be two pairs of genes for the synthesis of the alfa chain but only one pair for the synthesis of the beta chain.

Hemoglobins with reduced solubility:

Sickle-cell hemoglobin (HbS) is the major abnormal hemoglobin in this group. The 6th amino acid of the beta chain is valine instead of the normal glutamic acid of HbA. Other hemoglobins like HbC, HbD, HbE and HbJ have also slightly altered solubilities and cause mild degrees of anemia.

The erythrocytes in sickle-cell anemia are sickle shaped instead of being biconcave discs. The sickling is better demonstrated when the HbS is in the reduced state. When the oxygen tension is low, HbS molecules tend to aggregate to form filaments or tubular structures and precipitate from solution. Sickle cells have shorter life span than normal cells and are more easily lysed. Hence the anemia. The blood flow in the capillaries may be partially or completely blocked by the cells causing severe pains and damage to tissues. Erythrocytes of people with sickle cell trait contain both HbA and HbS. In those suffering from sickle cell disease, only HbS is present. The erythrocytes in sickle cell trait seem to be more resistant to malaria than normal.

Hemoglobins with altered oxygen affinity: Over 20 hemoglobins have been described, mostly named after the place they were first reported from-eg. Hb Chesapeake, Hb Kansas etc. The structural changes may involve the contacts between the alfa and beta chains, the DPG binding site or the heme pockets.

Some of the hemoglobins are readily oxidized (Fe^{++} converted to Fe^{+++}) to form methemoglobin (HbM). The condition is called methemoglobinemia. Five different types of HbM are known to exist, differing from normal hemoglobin in their ready convertibility to methemoglobin. This is on account of replacement of an amino acid (histidine or valine) in the heme pocket by tyrosine or glutamic acid.

Unstable hemoglobins: Some hemoglobins undergo in vivo oxidation and get precipitated to form 'inclusion bodies' or 'Heinz bodies'. Hb Hammersmith is one such example.

Thalassemia:

The condition is so named, because it occurs commonly in the Mediterranean countries (Thalassa means 'sea').

Normally the rates of synthesis of alfa and beta chains of hemoglobin are equal. In *thalassemia,* the rate of synthesis of one of the chains is low with the result, the other chain which is present in excess gets precipitated. In α-thalassemia, alfa chains are not synthesized adequately and hence beta chains are precipitated. In β-thalassemia, it is the reverse. The life span of the erthrocyte is much reduced and severe anemia develops. If alfa chains are not synthesized at all, a condition called *'hydrops fetalis'* is manifested. The fetus dies in utero or soon after birth.

Hemoglobin A_{1C} (Glycosylated form of Hemoglobin A_1):

In this, the n-terminal valine of the beta chain of HbA_1 is combined with 1-amino, 1-deoxy fructose. In normal persons, HbA_{1C} forms 3-5% of total hemoglobin. But, in diabetic subjects, it may form 6-15%. The level of glycosylated hemoglobin is an index of the level of blood glucose in the preceding several weeks and hence is a better measure of the diabetic state than a single blood sugar estimation.

Properties of Hemoglobin:

Acids hydrolyze and separate globin from heme. The heme is further oxidized to ferriheme. In the presence of HCL, this forms ferriheмechloride (hemin) which forms characteristic brown rhombic crystals readily identifiable under the microscope.

$$Hb + HCL \longrightarrow \text{Ferroheme} + \text{Globin}.$$

$$2\ \text{Ferroheme} + HCL + \tfrac{1}{2}0_2 \longrightarrow \text{Ferriheme chloride (hemin)} + H_2O.$$

If an alkali is used for hydrolysis, it forms ferrihemehydroxide or alkali hematin.

If ferriheme combines with globin, the compound formed is methemoglobin. Some drugs like nitrites, chlorates and ferricyanide oxidize hemoglobin in vivo and convert it to methemoglobin. The methemoglobin cannot function in the transport of oxygen.

Oxyhemoglobin

The most important property of hemoglobin is however its ability to readily form a complex with oxygen when exposed to high oxygen tensions and to dissociate equally readily and part with the oxygen when exposed to low oxygen tensions in the medium. The oxygen is only held loosely to the heme molecule by a redistribution of charges in the molecule. It is hence called oxygenation as distinct from oxidation.

$$Hb + O_2 \longleftrightarrow Hb\ O_2$$

At an oxygen tension (partial pressure) of 100mm Hg, the hemoglobin is 95-96% saturated with oxygen. This is what happens to it in the lungs where alveolar pO_2 is about 100mm Hg. In the arterial blood, the pO_2 is over 90 mm Hg and at this pO_2, the hemoglobin is still over 90% saturated and carries 19.6 ml of O_2 per 100 ml blood. In the tissues the O_2 tension is about 10% and the passage of blood through them makes the O_2 tension in it to fall to about 40% (venous blood). At this pressure Hb is only 75% saturated and retains only 12.6 ml oxygen and parts with the remaining 7 ml to the tissues.

The per cent saturation of Hb under various partial pressures of O_2can be plotted and are known as oxygen dissociation curves (see fig. 6-3). The dissociation is also found to depend on the pCO_2 tension. Under physiological conditions of 37°C temperature and pCO_2 of 40 mm Hg the dissociation curve is S shaped. It is relatively flat betwen pO_2 100 mm to pO_2 60 mm and shows over 90% O_2 saturation, thus ensuring adequate oxygenation of blood even under suboptimal, anoxic, conditions. The curve shows a sharper bend between pO_2 60 mm to 40 mm during which it falls from 90% to 75% O_2 saturation, thus facilitating rapid dissociation of Hb O_2. An increase in CO_2 over 40 mm will shift the curve to the right and vice versa.

The Hb can also combine, with other gases, and in some cases with greater avidity. Carbon monoxide has about 200 times as much affinity for Hb as O_2 and forms carboxyhemoglobin.

$Hb + CO \longrightarrow Hb\,CO$

Hence CO is highly poisonous if breathed. Prolonged breathing of pure O_2 is required to displace the CO from Hb.

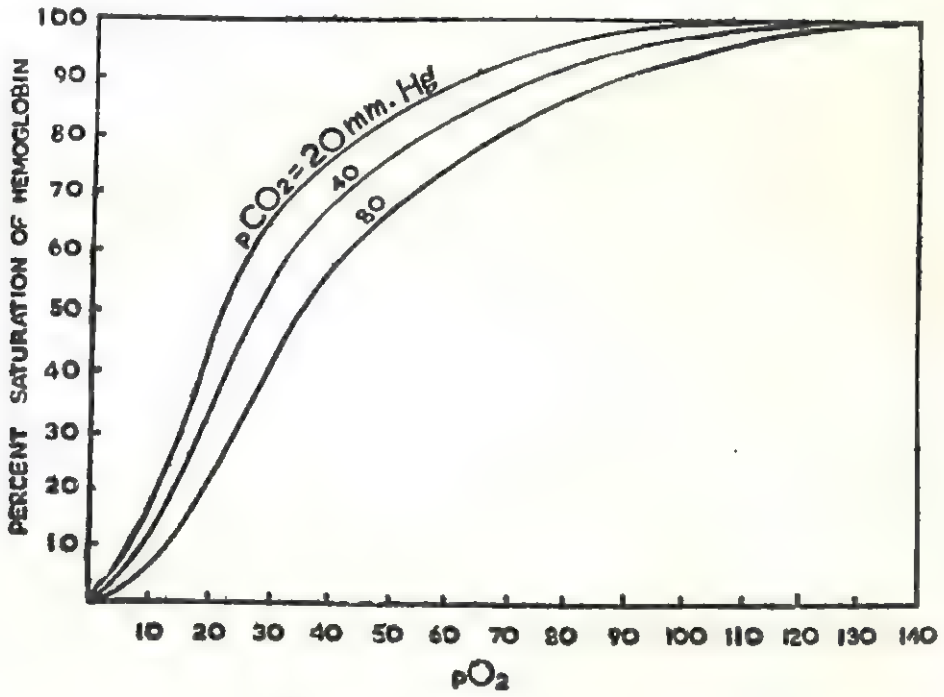

Fig. 6-3. Oxygen Dissociation Curves

Carbamino Hb: The hemoglobin can directly combine with CO_2 to form carbamino hemoglobin.

Buffering: Being a protein, it can also act as a buffer. This is true for reduced as well as oxyhemoglobin, both of which can be considered as weak acids, HHb and $HHbO_2$.

They can therefore form buffer systems thus.

$$\frac{Hb}{HHb} \quad \text{and} \quad \frac{HbO_2^-}{HHbO_2}$$

Also $HHbO_2$ is a stronger acid (pK = 6.6) than HHb (pK = 7.93). The conversion of the stronger acid $HHbO_2$ to the weaker HHb in the passage through the capillaries of the tissues will itself enable the weaker HHb to take up about 0.7 m eq. of H^+ from the tissues where it is liberated from $H_2CO_3 \longrightarrow H^+ + H\,CO_3^-$.

This is known as isohydric transport of CO_2 (without change in pH). THe H^+ is taken up by the reduced Hb and the HCO_3^- passes into plasma in exchange for chloride which enters the erythrocyte (on account of Donan membrane effect).

By the two mechanisms (isohydric transport and carbamino hemoglobin formation) hemoglobin helps in the transport of a major portion of CO_2 formed in the tissues.

Sulfhemoglobin: When oxyhemoglobin is exposed to H_2S, sulfhemoglobin is formed.

$$HbO_2 + H_2S \longrightarrow Hb\,S + O_2$$

This reaction is not reversible. Hence Hb S is no longer useful for transport of O_2.

Plasma: The chief solids of plasma are the proteins which are about 7-9 g per 100 ml. The individual proteins can be separated out from the mixture by different methods. 'Salting-out' methods employ different concentrations of salt solutions (e.g. ammonium sulfate, sodium sulfate and sodium sulfite) at which different proteins are precipitated and can be removed. More recently methods like electrophoresis and ultracentrifugation and ethanol fractionation (Cohn) have been developed.

Plasma Proteins: Using the early salting out techniques three proteins have been separated — albumin, globulins and fibrinogen. Globulins are precipitated by half saturation with ammonium sulfate whereas albumin is precipitated only on full saturation. Fibrinogen is precipitated by 1/5th saturation with ammonium sulfate. Among the globulins, there is a fraction which can be precipitated on 1/3 saturation with ammonium sulfate. This is termed the 'euglobulin' (true globulin) and the rest called 'pseudoglobulin' (false globulin). Euglobulin is insoluble in distilled water but soluble in dilute salt solutions, say NaCl; but pseudoglobulin is soluble even in distilled water.

Howe fractionated serum proteins by using different concentrations of sodium sulfate. Cohn used varying concentrations of ethanol at low temperature to separate out fractions of proteins which he called Fraction I, II and so on. Each fraction is itself a mixture, but contained one of the proteins predominantly. Because the solvent can be readily removed by evaporation and the mild procedures used in the separation do not cause denaturation, Cohn's method is useful for obtaining purified plasma proteins on a large scale for therapeutic purposes.

Electrophoresis: Tiselius (1937) developed this method of analyzing plasma proteins. It is based on the principle that in solutions whose pH is above or below that of the isoelectric points of a mixture of proteins, the proteins will migrate in an electric field to the anode or cathode at different rates, the highest rate of migration being for the protein whose isoelectric pH is farthest removed from the pH of the solution and whose molecular size is smallest. This separation can be obtained in a free solution kept in a U-tube by passing a current between two electrodes inserted in each limb of the U. After running the current for several hours, the proteins which collect at different portions of the limbs of the U are analyzed by suitable optical methods and photographing. A pattern of peaks is obtained as shown in

Fig. 6-4, each peak representing one protein fraction. The protein fractions can be estimated quantitatively by measuring the areas under the curves for each peak.

Similar results can be obtained by using a filter paper strip to support the fluid instead of using an U-tube. The filter paper is held between two compartments containing a buffer of suitable pH and the protein mixture (say plasma) is applied as a small dot or as a line across one end of the filter paper. The current is passed through the filter paper by electrodes dipping into the two buffer chambers. The proteins migrate at different rates to the other end of the filter paper and in several hours time, they get separated out sufficiently from one another to be identified by simple staining methods (fig. 6-4) and may be estimated by suitable methods of densitometry of the stained paper or elution of each stained portion into suitable solvents and estimating colorimetrically. Instead of paper, several other supporting media like cellulose acetate, starch etc., can be used for such electrophoresis.

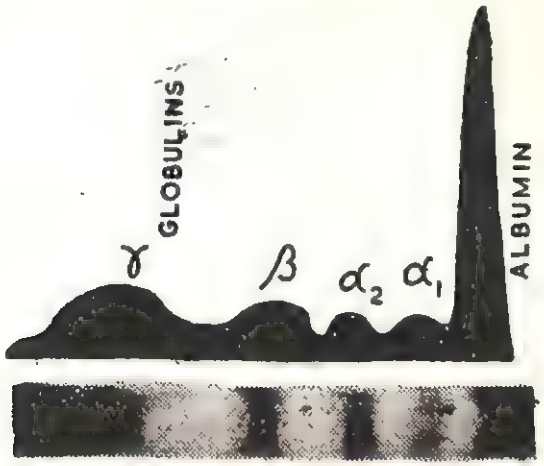

Fig. 6-4. Serum Proteins — Electrophoretic Pattern

By such methods the serum can be separated into a number of fractions — albumin and three different types of globulins, α, β and γ. Depending on the sensitivity of the method used, the α globulin can be separated into an α_1 and α_2 fractions and β into a β_1 and β_2 fractions. If plasma is used instead of serum, the fibrinogen fraction is seen as a band between the β and γ globulin peaks.

Using modern analytical techniques, more than eighty proteins can be identified in plasma. Many of them occur only in trace amounts and are either enzymes or transport proteins. Possibly several other proteins may exist in plasma which cannot be identified by the presently available methods.

The proteins which can be readily identified by routine laboratory methods and which are present in more than trace amounts are listed in Table 6-1.

Table 6-1

Total proteins	.. ..	6.3 –7.8	g/100 ml
Albumins	.. ..	3.2 –5.1	"
Alfa$_1$ globulins	.. ..	0.06–0.39	"
Alfa$_2$ globulins	.. ..	0.28–0.74	"
Beta globulins	.. ..	0.69–1.25	"
Immunoglobulins			
(gamma globulins)	I_gA	0.15–0.35	"
	I_gG	0.80–1.80	"
	I_gM	0.08–0.18	"
Fibrinogen	.. ..	0.20–0.40	"

Albumin: It is the most abundant and fairly homogeneous protein of plasma with a molecular weight of 69,000. It has a low isoelectric pH (4.7). It migrates fastest in electrophoresis and precipitates last in salting out or alcohol precipitation methods.

It contains about 580 amino acids and 17 interchain disulfide bonds. It is a simple protein (globulins are mostly glycoproteins). The molecule is ellipsoidal in shape (30 x 150 Å), and its solutions have low viscosity. It contributes 70-80% of the osmotic pressure of plasma proteins. It undergoes constant exchange with the albumin present in extracellular spaces of muscle, skin and intestines. It also helps in the transport of several substances like free fatty acids, bilirubin, Ca^{++}, and steroid hormones. Drugs like sulfonamides, penicillin and aspirin also bind to albumin.

Alfa Globulins: They are glycoproteins and are further classified into α_1, α_2 etc., depending on their electrophoretic mobility. α_1-Acid glycoprotein has some structural resemblance to immunoglobulins. Alfa fetoglobulin is present in high concentrations in fetal blood during mid-pregnancy. Normal adults have less than 1 μg/100 ml. It may increase during pregnancy. Some of the alfa globulins act as inhibitors of coagulation and also inhibit some enzymes like trypsin and chymotrypsin. There is a 'retinolbinding' protein and also a protein which, together with prealbumin, binds thyroxine. Ceruloplasmin is a copper containing alfa$_2$ globulin and has eight sites for binding copper ions. It functions as a ferredoxidase and helps conversion of Fe^{++} to Fe^{+++}, which can be incorporated into 'transferrin'. It also serves to transport Cu^{++}. In Wilson's disease, plasma ceruloplasmin levels are markedly decreased and copper levels of liver and brain are increased resulting in damage to those tissues. Haptoglobulins account for a fourth of the α_2 globulins. They can form complexes with hemoglobin. This is a mechanism which prevents the urinary loss of hemoglobin released when erythrocytes break down.

Beta Globulins: Lipoproteins form an important constituent of this group. Transferin, the iron binding protein, is a beta globulin. Hemopexin is another which binds heme and prevents its excreton. C– Reactive protein is also a beta globulin. It is present in concentrations of less than 1.0 mg/100 ml in adult blood. It precipitates with a group C polysaccharide of Pneumococcus in the presence of Ca^{++}. Its levels in plasma are increased in acute infections.

β_2-Microglobulin: It has a low molecular weight and is excreted in urine. It is present in urine to the extent of only 0.01 mg/100 ml. It has also close structural resemblance to immunoglobulins.

Immunoglobulins (Gamma globulins)

The immune system consists of two entities —
1. The Cellular Immune System: This is mediated by the T cells (thymus derived lymphocytes) and is active against microorganisms, fungi, parasites, foreign tissues etc.
2. The Humoral Immune System: This is mediated by the B cells, (lymphocytes *not* derived from thymus), which secrete 'antibodies' or immunoglobulins when exposed to foreign substances called 'antigens'.

The immune system is spread diffusely throughout the body and consists of 10^{12} cells in the spleen, liver, bone marrow, thymus, lymph nodes and in the circulating blood. The cells have a mass of 2 Kg and produce about 60 grams of protein for the immune system. In malnourished individuals, particularly in protein malnutrition, the immunity is low and the susceptibility to infection is high.

The different immunoglobulins can be broadly classified into three major classes — I_gG, I_gA and I_gM — and two minor classes — I_gD and I_gE. They are distinguished by their differences in sedimentation rates on ultracentrifugation, immunoelectrophoretic behaviour and response to antigen stimulation. All of them contain two identical light chains (L) and two identical heavy chains (H) held together as a tetramer, (L_2H_2). In the light chain, the half towards the c-terminal is the *constant region* (CL) and the half towards the n-teminal is the *variable region* (VL). Similarly, about 3/4 of the heavy chain towards the c-terminal is the constant region and 1/4 of the heavy chain towards the n-terminal is the variable region (CH and VH respectively). The constant region of the heavy chain is further subdivided into three regions — C_H1, C_H2 and C_H3.

The n-terminal VL and VH regions (or domains) are the ones which determine the specificity of the antibody for the antigen and take part in binding the specific antigen. (See Figs. 6-5 and 6-6). Digestion with papain will produce two antigen binding fragments (Fab) and one crystallizable fragment (FC). The cleavage occurs between C_HI and C_H2 regions, which is called the *hinge region*.

The L chains are either kappa (K) or lambda (λ) and an immunoglobulin contains only one of them. The H chains can be any one of the five — gamma (γ), alfa (α), omega (ω), delta (δ) or eta (ξ). The classification of the immunoglobulins is based on the H chains. Delta and eta chains have four CH domains instead of only three in the others. The main characteristics of the five classes of immunoglobulins are tabulated in Table 6-2.

Table 6-2

I_g type	H chain	L chain	Carbohydrate content
I_gG	gamma	kappa or lambda	4%
I_gA	alfa	"	10%
I_gM	omega	"	15%
I_gD	delta	"	18%
I_gE	eta	"	18%

It can be seen from above that all immunoglobulins are glycoproteins containing varying amounts of carbohydrate. The H chains have a molecular weight ranging from 50,000 to 75,000. The L chains have a molecular weight about 23,000. While I_gG occurs as such, I_gA and I_gM may occur as polymers containing two or more identical subunits. The L and H chains are synthesized separately in the B cell and later assembled together. At least three genes take part in the synthesis of the L chain and four genes in the synthesis of the H chains. Man has the ability to synthesize antibodies to at least a million antigens.

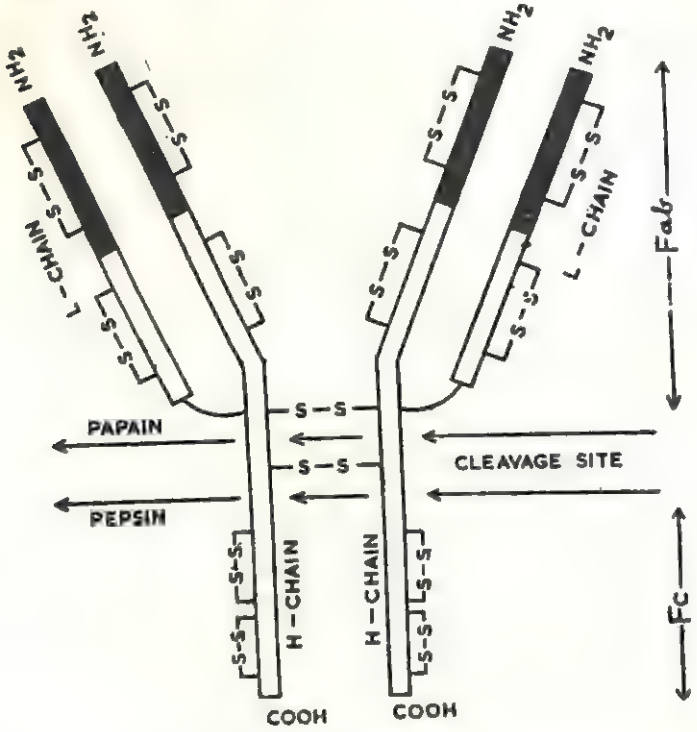

Fig. 6-5. Structure of I_gG

Diagrammatic representation of the structure of I_gG showing heavy (H) and light (L) polypeptide chains joined by S-S bonds. The amino (NH_2) and carboxy (COOH) terminal portions of the peptide chains are also indicated. Shaded areas at the amino end of each chain outline the sites of variable amino acid compostion; the unshaded areas, those which appear to be constant in amino acid sequence when the structures of different antibody molecules are compared. The 2 carbohydrate (CHO) units which are present in I_gG are indicated. The sites of cleavage by proteolytic enzymes such as trypsin, to form 2 Fab fragments and one Fc fragment, are also shown in the diagram. Fab means "Antigen Binding Fragment". Fc means "Crystallizable Fragment".

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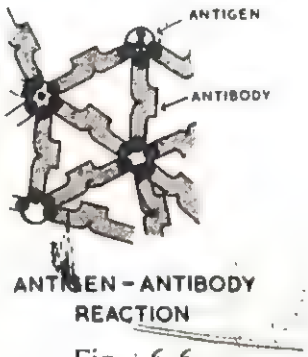

Fig. 6-6.

Biological role of immunoglobulins

I_gM: This is the first antibody formed in response to an antigen. It has multiple, though weak, binding sites for antigens and can agglutinate cells with surface antigens. It is only slightly permeable into interstitial fluids and is impermeable to placental barrier. It is therefore mainly found in the plasma.

I_gG: This is the next immunoglobulin formed in response to antigenic stimulation. Quantitatively, it is present in the largest amounts in plasma and its rate of synthesis is the highest (over 2 grams per day). It can enter the interstitial fluid and also cross the placental barrier and can provide immunity to the fetus. It readily binds to the antigen and also stimulates the complement system.

I_gA: This is produced still later. It gets concentrated at sites of entry of antigens into the body — e.g. the digestive and respiratory tracts. It is the chief constituent of the colostrum milk (milk secreted soon after child birth) and provides protection to the gastrointestinal tract of the new born infant against microbial infection through gastrointestinal tract.

Antibodies can be either *monoclonal* or *polyclonal*. The monoclonal antibodies are highly specific and bind a single antigenic determinant of an antigen. They are produced by cells derived from a single clone. Polyclonal antibodies are derived from multiple clones and are mixtures of several antibodies formed by different types of immunoglobulins.

Immunity:

Immunity can be either active or passive.

Active immunity is acquired as a result of exposure to foreign cells or macromolecules either naturally or artificially (e.g. smallpox vaccination, cholera inocculation). Immunity against smallpox, polio, typhoid and measles is of this type.

Passive immunity is obtained by injecting antibodies (prepared in another animal or human being exposed to the antigen earlier) into the susceptible individual. Injections of diphtheria and tetanus antitoxins are of this type. Immunity offered by this method is transient and short lived compared to active immunity.

The Complement System

This comprises of a group of proteins in the blood which complement the functions of the antibodies in eliminating antigens. They act by (i) enhancing capillary permeability and passage of phagocytic leukocytes to the site of antigen; (ii) stimulating phagocytosis and (iii) lysing microorganisms and tissue cells foreign to the organism. The components of the complement system exist in the plasma in an inactive (zymogen) form and have to be activated first. The proteins participating in the pathway can be broadly classified into three groups:

1. The C_1 complex forms the recognition unit and binds to specific antigen-antibody complex on the cell surface.
2. The C_2, C_3 and C_4 form one unit called the activation unit. The binding of the C_1 unit to the antigen-antibody complex triggers on this unit to function.
3. C_5, C_6, C_7, C_8 and C_9 form the membrane attack unit. This is activated by the activation unit formed in (2) above and attacks the cell membrane and causes death of the cell. Detailed chemistry and functions of these several components had been worked out but are beyond the scope of this book.

Sometimes, even without the formation of the antigen-antibody complex and full activation, the complement system may directly recognize the invading microorganisms and act on them after the initial stages of complement activation only. This is referred to as the '*alternative pathway*'.

Haptens: Small molecules, which are poor antigens by themselves, when coupled with a carrier protein molecule, become potent antigens. Such small molecules are called 'haptens'. Usually they are aromatic, charged molecules.

Fibrinogen: This is considered in detail under 'coagulation'.

Site of formation of plasma proteins:

Experiments described as 'plasmapheresis' (wherein an animal is bled repeatedly, the plasma is separated from the cells by centrifugation, and the cells are reinjected suspended in saline) are performed whereby the plasma protein level is very much reduced. It is possible in such an animal to find out the amount of each of the plasma proteins sythesized by finding out the amount of plasma that has to be removed to keep the level of that constituent constant in the animal. This amount of particular protein is the basal output by that animal. It is found that this basal output is reduced to about one tenth the normal in an animal where the liver is short circuited from circulation by anastomosing the portal vein to venacava (surgically known as ECK fistula). In cirrhosis of the liver (where the circulation through liver and the function of the liver are grossly deranged) the plasma protein levels are very much decreased. The proteins involved in all these conditions are the albumin, alfa and beta globulins and the fibrinogen. The gamma globulin actually increases. By these and other experiments, it is established that liver is the sole source of fibrinogen and albumin. It is also the source for most of the α and β globulins. The gamma globulins are however derived mainly from the plasma cells and lymphoid tissues (reticuloendothelial system). Alterations in plasma proteins are of diagnostic value. In most conditions involving changes in plasma proteins, there is a decrease in albumin and an increase in globulins (mainly gamma) leading to an alteration of the ratio albumin/globulin (normmally 4.5/2.2 or 2:1 by salting out methods; and 3.8/2.9 or 1.3:1 by electrophoresis method). In severe cases, the ratio becomes reversed (A/G becomes 1/2 instead of 2/1). These alterations occur in diseases of kidney due to loss of protein (mainly the low molecular weight albumin) and in chronic infections due to increased amount of gamma globulin (antibody formation) and in diseases of the liver (due to failure to synthesize albumin).

Functions of Plasma Proteins:

1. *Fluid Exchange:* Plasma has an osmotic pressure of about 6.5 atmospheres. A small portion of this (25 mm Hg i.e. about 1/30 atmospheric pressure) is contributed by plasma proteins. The other extracellular fluids have much less protein content (about 10 mm Hg). The electrolyte content being the same, this difference in protein osmotic pressure helps the plasma to draw fluid from the extracellular space. This occurs at the venous end of the capillary. At the arterial end of the capillary, the hydrostatic pressure in the capillary which is about 30 mm Hg will drive the fluid into the tissue spaces where the pressure is only 10mm Hg. An effective exchange of fluid between tissue spaces and plasma is thus maintained (see Figure 6-7).

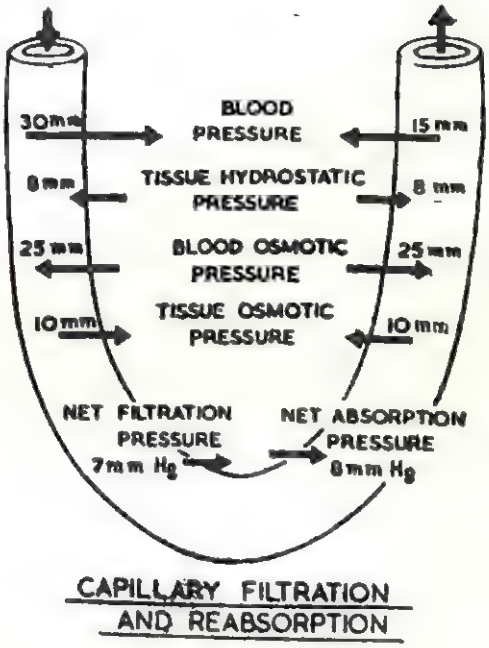

Fig. 6-7

In this function, albumin plays a greater role than globulins. Being a smaller molecule, it exerts a higher osmotic pressure, weight per weight, compared to the heavier globulins. One gram of albumin per litre of serum exerts 5.54 mm Hg. pressure, whereas one gram globulins exert only 1.43 mm Hg.

2. *Buffering Action:* At pH 7.4 of blood, plasma proteins act as weak acids, and combine with cations, mainly sodium. This accounts for about 16 m.eq. of sodium/litre (out of 143 m.eq. per litre).

3. *Reserve Protein:* The plasma protein can be taken up by tissues and used for the building up of tissue proteins and vice-versa.

4. *Transport:* Insoluble substances like bilirubin, steroid and other hormones, fatty acids and other lipids and metals are transported in plasma in loose combination with plasma protein.

Occasionally inherited deficiencies occur in certain of the plasma proteins e.g. afibrinogenemia and agammaglobulinemia. These conditions will be associated with bleeding disorders in the one case and lowered resistance to infection in the other.

Macroglobulinemia: I_gM (19S) fraction is much increased. Heavier macroglobulins (24-40S) may also occur. The condition occurs in neoplastic diseases, collagen disorders, chronic infections, amyloidosis and hepatic cirrhosis.

Multiple Myeloma: An abnormal protein, 'Bence Jones protein' occurs in blood and urine of people suffering from this condition. It has a low molecular weight (45,000; 3.5S), and

hence readily escapes through the glomerular filter into urine. It is possibly a dimer of the L-chains. It is easily identified in the urine by a simple test. On heating the urine to 50°-60°C. Bence Jones protein is precipitated but when heated further, it dissolves again.

Cryoglobulins: These proteins are coagulated when the plasma or serum is cooled to very low temperatures. Traces are present even in normal individuals. They are increased in rheumatoid arthritis, lymphocytic leukemia, multiple myeloma and lymphosarcoma. The molecular weight varies from 165,000-600,000.

In addition to the chief proteins described above which are present in appreciable amounts, traces of several other proteins are present which are detectable mainly by their action and isolated with great difficulty by use of highly sophisticated methods. This group will include dozens of enzymes and several factors concerned with coagulation or clotting of blood.

Enzymes like amylase, lipase, transaminases and phosphatases are present in blood. They also show quantitative variations in disease and are of diagnostic importance. The clotting factors are considered separately.

COAGULATION OF BLOOD

When blood is drawn and left to itself in a container outside the body, it forms a solid mass in a very short time. This is known as clotting of blood. In the course of a few hours a clear straw colored liquid exudes from the red mass. This is called serum as distinguished from plasma which is obtained by centrifuging a sample of blood treated with an anticoagulant and collecting the clear supernatant fluid.

Howell originally described clotting to consist of 3 phases:

Stage I: Thromboplastin is liberated from injured tissue or shed blood.

Stage II: The thromboplastin in the presence of ionic calcium acts on prothrombin, a protein present in blood plasma and converts it to thrombin.

Stage III: The thrombin in turn acts on fibrinogen, the soluble protein present in plasma and converts it to fibrin which is insoluble and precipitates out as a network of elongated thread like fibres enmeshing the cellular and liquid components in the mesh work and giving it a solid appearance.

Subsequently the fibres shrink and the mesh becomes closely knit, still holding the cellular elements, but exuding out the liquid with its dissolved proteins and other organic and inorganic constituents. This fluid is the serum.

This simple description involving only 4 principal factors — thromboplastin, calcium, prothrombin and fibrinogen — is now complicated by the discovery of several new factors and by an increasing insight into the detailed mechanisms involved in the clotting process.

Arrest of bleeding is called '*hemostasis*' and occurs in 4 phases.

1 Vasoconstriction of the injured vessels, thereby reducing the blood supply to the area.

2. Formation of a loose plug of platelets — a *white thrombus* — at the site of injury. Collagen fibres exposed at the site of injury act as scaffolding on which the platelets get

adherent and undergo disruption releasing serotonin, epinephrine, prostaglandins and metabolites like thromboxane and ADP. These products increase the adhesiveness of the platelets and more platelets are deposited to form the platelet plug. Determination of bleeding time measures these two phases.

3. Formation of the '*red thrombus*' (blood clot).

4. Partial or complete dissolution of the clot (before which the injury would have healed).

If a clot forms in an intact blood vessel without an apparent injury, it is called a *thrombus*. Several factors have been described (thirteen to date) which have a role in the clotting process. They are designated by a common name as well as by a number. Many of them exist in circulation in an inactive form and have to be activated to enable their participation in the clotting process. Activation usually involves proteolytic removal of a segment or segments from the molecule. In fact, many of the factors are themselves *serine proteases* and help in activating each other, in a cascade like fashion. The numerical notation, the common name and some of the more important properties of the factors are listed below.

Factor I, Fibrinogen: Soluble glycoprotein molecular weight 340,000. Made up of 6 polypeptide chains — two alfa, two beta and two gamma chains. The alfa, beta and gamma chains are linked lengthwise by –S–S– linkages. Two such chains are linked again by –S–S– bridges. At the junction point, there is a swelling called the *disulfide knot*. The terminal portions are helical structures and also form two swellings, one at each end. The head ends carry a high negative charge due to presence of aspartate, glutamate and tyrosine residues in those portions. This prevents these fibres from coming together to form a polymeric structure (the clot). They also keep the fibrinogen molecules in solution. Fibrinogen is synthesized in the liver.

Factor II, Prothrombin: This also is synthesized in the liver. Molecular weight, 72,000. It is a single chain glycoprotein. At one end, it has upto 14 molecules of gamma-carboxyglutamic acid (GLa). Vitamin K is required for their incorporation into the molecule and a deficiency of the vitamin therefore causes a deficiency of prothrombin. Factor Xa (active Stuart Factor) converts prothrombin to thrombin by proteolysis at two points. Thrombin is the active form and can remove, by proteolysis, small peptides from the n-terminal ends of the alfa and beta chains of fibrinogen which contain the negative charges. The remnant molecules, now called '*fibrin*' (monomeric form) are no longer soluble. They aggregate together and polymerize in the presence of calcium ions (factor IV) to form the clot (see Fig. 6-8).

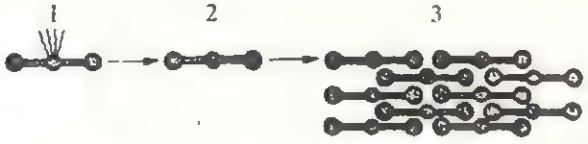

Fig. 6-8 Conversion of fibrinogen to fibrin

1. Fibrinogen molecule
2. Fibrin monomer formed by removal of fibrinopeptide
3. Fibrin lattice formed by aggregation of the monomers

Thrombin not only brings about proteolysis of fibrinogen; it also converts Factor XIII (Laki-Lorand factor) to its active form – XIIIa. This is a transglutaminase and causes establishment of cross linkages beetween gamma-carboxyl groups of glutamine of one fibrin polymer with the eta-amino groups of lysine of a neighbouring fibrin polymer, thus strengthening the clot and also causing *clot retraction.*

Factor IV, Calcium ions: Ionic calcium is required for several stages in the clotting process and is mentioned at the appropriate places.

Factor V, Labile Factor, Proaccelerin, Accelerator (AC) Globulin: It does not have any enzyme properties. It is activated to Va (formerly called Factor VI) by thrombin. In turn Factor Va is necessary for activating Factor X (Stuart factor) in the intrinsic system.

Factor VII, Proconvertin; Serum Prothrombin Conversion Accelerator (SPCA): This is an accessory protein activated to VIIa by thrombin. In turn, it activates Factor X to Xa in the extrinsic system.

Factor VIII, Antihemophilic Globulin (AHG); Von Willebrand Factor: Also an accessory protein activated to VIIIa by thrombin. It enhances the rate of activation of Factor IX (Christmas Factor).

Factor IX, Christmas Factor: It contains glutamic acid carboxylate residues just like prothrombin and is activated to IXa by Factor XIa. It also converts the inactive Factor X to the active form Xa. This is a very slow process, but is enhanced 500 fold by the presence of Factor VIII or VIIIa.

Factor X, Stuart Factor: This is the junction point for the extrinisc and intrinsic pathways. It is a serine protease and contains GLa residues. They help in calcium binding with acidic phospholipids of platelets. Factor X is converted to Xa in the extrinsic pathway by VIIa. Once some Xa is formed, it autocatalytically converts large amounts of X to Xa.

Intrinsic Pathway: This commences with exposure of prekallikrein, a high molecular weight kininogen, factor XII and factor XI to an active surface — say collagen, in vivo and glass or kaolin, in vitro. Kallikrein will convert XII to the active XIIa by proteolysis. XIIa releases bradykinin from high molecular weight kininogen and activates XI to XIa. All these, together with factors IXa, VIII or VIIIa convert factor X to Xa by proteolysis. Ca^{++} and phospholipids are also required for the conversion.

Factor XI, Plasma Thromboplastin Antecedent, PTA: Activated to XIa by Factor VIIa. The XIa in turn activates Factor IX to IXa.

Factor XII, Hageman Factor: It is activated to XIIa by contact with glass or by kallikrein system.

Factor XIII, Fibrin Stabilizing Factor: It is the zymogen form of a transglutaminase and is activated to the zymase form, XIIIa, by thrombin. It helps consolidation of the clot by cross linkages between the gammacarboxyl groups of glutamine and the eta-amino groups of lysine.

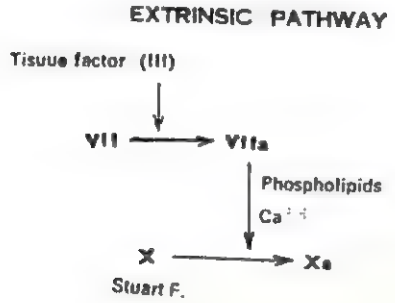

Fig. 6 - 9 a

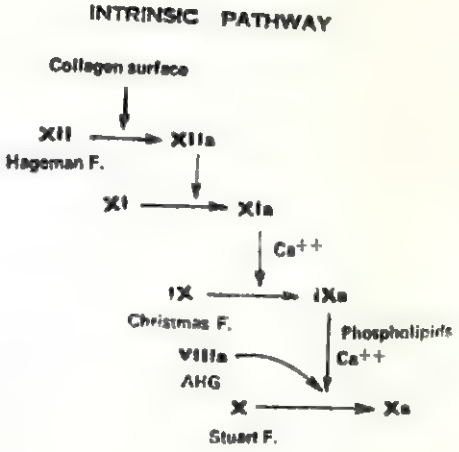

Fig. 6 - 9 b

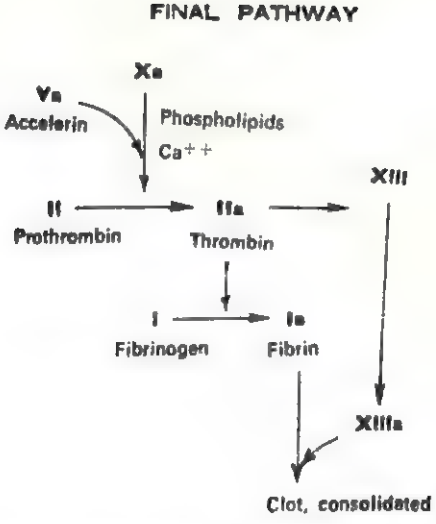

Fig. 6 - 9 c

Fig. 6-9a: Extrinsic pathway of blood coagulation
Fig. 6-9b: Intrinsic pathway of blood coagulation
Fig. 6-9c: Final pathway of blood coagulation: Please note that the end product in both the intrinsic and extrinsic pathways is Factor Xa (active Stuart Factor). This forms the starting point for the Final pathway.

Factor XIV, Protein C: It is a zymogen of a protease, which, on activation by thrombin to XIVa, inactivates factors V and VII by proteolysis.

Pre-Kallikrein: On conversion to Kallikrein by contact, it activates Factor XI and XII.

High Molecular Weight Kininogen: Accessory protein factor for activation of factors XI and XII.

Plasminogen: This is the zymogen form of Plasmin. It is activated to plasmin by tissue factors and brings about fibrinolysis.

Factor III, Tissue Factor, Thromboplastin: Accessory protein from tissue, in the presence of phospholipids, enhances the extrinsic pathway of clotting.

What triggers clotting?

Extrinsic Pathway: This produces rapid coagulation. Thromboplastin, a lipoprotein tissue factor, comes into contact with blood, on injury to endothelium or tissue. It activates Factor VII present in the blood to VIIa. The factor VIIa formed will in turn activate factor X to Xa.

Intrinsic Pathway: The initial reaction involves the exposure of Factor XII to the negatively charged surface of collagen or activated platelets. Factor XII is then hydrolyzed to XIIa which now activates Factor IX to IXa in the presence of Ca^{++}. Factor IXa, in the presence of Factor VIII or VIIIa, platelet phospholipids and Ca^{++} will activate Factor X to Xa.

Final Pathway: In the intrinsic or extrinsic pathway, the activation of factor X to Xa is the end reaction. Thus formation of factor Xa is the junction point for the two pathways.

Factor Xa, in association with Va and Ca^{++} ions converts prothrombin to thrombin. Thrombin will now convert fibrinogen to fibrin monomers by proteolytic removal of fibrinopeptides from fibrinogen. Simultaneously, it activates factor VIII to VIIIa, an active transglutaminase. The fibrin monomers get cross linked to form the fibrin polymer or the clot.

A summary of the intrinsic and extrinsic pathways of coagulation and the final pathway is presented in Fig. 6-9,a,b,c.

Common Tests employed to identify Coagulation Disorders

Bleeding Time: It is an index of the effectiveness of the platelets in forming a plug to arrest bleeding from a needle puncture.

One Stage Prothrombin Time: Tissue factors (Thromboplastin) are added to plasma and the time taken for clotting is measured. This is a test for the efficiency of the extrinsic and final pathways of clotting. Abnormalities can occur due to deficiency of prothrombin, factors V, VII or X. If the time is restored to normal by addition of small amounts of normal serum (which contains factors VII and X), the deficiency must be in one of these two factors. If it is not restored to normal by addition of normal serum, then the deficiency is in prothrombin or factor V.

Clotting Time: Blood drawn with minimum injury to tissue is placed in a test tube and allowed to clot. (Tissue factors are eliminated by avoiding injury to tissues). On contact with glass, the intrinsic system is set into action, and the time taken for the formation of the clot is a measure of the effectiveness of the intrinsic and final pathways. It depends on all the coagulation factors except factors VII and XIII.

Inhibitors of clotting:

There are several proteins present in plasma which act as inhibitors of the proteases involved in blood clotting. *Antithrombin III* is the chief amongst them and inhibits all serine proteases including trypsin, chymotrypsin, plasmin, thrombin, factors IXa, Xa, XIa and XIIa.

Heparin acts as a very effective anticoagulant mainly by activating antithrombin III.

Coumarin group of drugs (eg. *Dicumarol)* act by interfering with vitamin K dependent carboxylation and conversion of glutamic acid to gamma-glutamyl carboxylate (GL to GLa). The production of prothrombin, Factors V, VII, IX and X is decreased. Thus all stages of coagulation are inhibited.

Oral contraceptives may diminish Antithrombin III activity and thus favour intravascular thrombotic phenomena.

A deficiency of Antithrombin III may be also inherited as an autosomal dominant.

Protein C: Extenison of the blood clot beyond the point where it is required to cause hemostasis is prevented by trapping all the activated factors within the initial blood clot formed, so that they are no longer free to act on the circulating blood. In addition, there is a specific protein in the plasma - *Protein C* - which is activated by thrombin. The activated protein C destroys Factors Va, VIIIa and Xa in vitro. It may exert the same action in vivo also.

Fibrinolysis

A proenzyme present in plasma - *plasminogen* - is activated to *plasmin* by kallikrein. The plasmin formed hydrolyzes fibrin to a number of small, soluble peptides. The clot is thus dissolved. Protein C may also play a role in the conversion of plasminogen to plasmin.

Bradykinin is one of the byproducts of the action of kallikrein on large molecules in the plasma called *kininogens* (M.W. 50,000 to 250,000). Bradykinin is a small peptide with only 9 amino acids. It dilates blood vessels, increases their permeability, constricts smooth muscle and is mainly responsible for causing intense peripheral and visceral pain by stimulating the pain receptors.

Complement:

The complement system (see under plasma proteins) is also triggered into action on formation of Factor XIIa.

Anticoagulants:

In Vitro: Blood is prevented from clotting in vitro mainly by preventing access to calcium ions. Removal of Ca^{++} by addition of oxalate, citrate, ion exchange resins and EDTA (ethylenediamine tetra-acetate) will either precipitate calcium as an insoluble salt or convert it into a non-ionizable salt and thus prevent the coagulation process.

Other constituents of blood

The other important chemical constituents of blood are listed in the table 6-3

TABLE 6-3

Clinically Important Constituents of Human Blood

		Per 100 ml.
Hemoglobin	...	12–17 gm.
Plasma proteins	...	6.5–8.0 gm.
Glucose (true glucose), fasting	...	60–90 mg.
Non-protein nitrogen	...	30–40 mg.
Urea	...	12–40 mg.
Creatinine	...	0.7–1.5 mg.
Uric acid	...	3.0–5.0 mg.
Cholesterol, serum, total	...	150–250 mg.
Cholesterol, serum, free	...	50–70 mg.
Cholesterol, serum, ester	...	100–180 mg.
Sodium, serum	...	320–340 mg. or 138–148 milliequivalents/ litre
Potassium, serum	...	16–22 mg. or 4.2–5.5 m.eq./litre.
Chlorides, serum	...	355–390 mg. or 100–110 m.eq/litre.
Calcium, serum	...	9–11 mg. or 4.8–5.2 m.eq./litre.
Inorganic phosphorus, serum	...	3–5 mg. or 1.4–2.7 m.eq./litre.
Iodine, serum	...	8–15 microgrammes.
Bilirubin, serum	...	0.2–0.8 mg.

(Where specifically not stated, the values are for 100 ml. of whole blood).

CEREBROSPINAL FLUID

It is formed by the choroid plexus and secreted into the lateral ventricles from where it enters the third and fourth ventricles and is finally absorbed through the subarachnoid space. Its volume is about 5 ml. in the new born and reaches 100 to 150 ml. in the adult.

Under normal conditions, secretion of C.S.F. is slow and may not exceed 100-150 ml. a day. If a lumbar puncture is performed with the subject in the reclining (horizontal) position, the fluid exerts the same pressure as on cisternal puncture and is about 110-130 mm. water (Ringer saline) or 7-10 mm. mercury. Compression of the jugular vein causes a rise in the CSF pressure (Queckenstedt sign.)

The main function of CSF is to provide a protective water jacket around the brain and spinal cord, and to enable alterations in the volume of these viscera without causing their compression in the rigid bony encasement. Other functions like nutrition and excretion are only incidental.

TABLE 6-4

Concentrations of Constituents in Cerebrospinal Fluid and in Serum of Normal Persons

Constituent	*Serum*	*CSF*
Glucose, mg/100 ml.	55–80	55–80
Urea nitrogen, mg/100 ml.	6–23	6–23
NPN, mg/100 ml.	20–30	20–30
Uric acid, mg/100 ml.	3–5	0.6–0.7
Protein, mg/100 ml.	6,500–8,500	20–40
Sodium, m. eq./litre	138–148	142–150
Potassium, m.eq./litre	4.2–5.5	2.3–3.2
Magnesium, m.eq./litre	1.6–2.1	2.5–3.0
Calcium, m.eq./litre	4.8–5.2	2.3–2.8
Chloride, m.eq./litre	100–110	120–130
Bicarbonate, m.eq./litre	24–29	24–29
Bilirubin, mg/100 ml.	0.2.–1.0	0
pH	7.4	7.4

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Composition of the CSF: It can be considered to be an ultrafiltrate of the plasma. Protein is present only in trace amounts. Lipids and bilirubin are absent. Glucose, urea and other organic constituents have about the same concentration as in plasma: pH is also same as in plasma. The calcium concentration is that of the diffusible portion in plasma (2.3-2.8 m.eq./litre). The concentrations of sodium and chloride are determined by the Donnan membrane equilibrium phenomenon and are somewhat higher than in plasma.

The concentrations of the more important constituents of CSF are presented and compared with those of serum in Tab. 6-4.

The magnesium level is nearly double that in plasma; potassium and inorganic phosphate, a little more than one half. The final composition of the CSF is dependent on two factors – (i) secretion of an ultrafiltrate by the choroid plexus and (ii) addition and removal of constituents by the meninges and nervous tissues. The prolonged contact with the meninges and the nervous tissues results in profound alterations in the secretion. Glucose, amino acids, phosphate and potassium are all taken up and magnesium is added.

Variations in disease:

Protein: Normal is 20–40 mg.%. It is higher in the spinal fluid than in the cisternal or ventricular fluids. The protein is mainly albumin with very little globulin and no fibrinogen. The protein content is increased in case of hemorrhage into the CSF (*eg.* subarachnoid or cerebral hemorrhage) A fresh hemorrhage will turn the fluid red. An old hemorrhage imparts a yellow color (xanthochromia) due to formation of bile pigments.

Protein content is also increased in inflammatory diseases like menigitis, encephalitis and myelitis and in tumors of the spinal cord or meninges. With the increase in protein concentration, there is a rise in globulin and fibrinogen content. If the latter is increased sufficiently, there may be spontaneous clotting in the CSF sample.

Cells: Normally the CSF contains no cells or a maximum of 5 cells per c.mm. They are exclusively lymphocytes. But in inflammatory conditions mentioned above, they will increase in numbers, with the polymorphs predominating.

Glucose: It is normally 55-80 mg.%. Fluctuations in blood glucose levels will elicit a slow alteration in CSF glucose due to sluggish circulation of the CSF. In encephalitis and poliomyelitis the CSF glucose is normal or slightly elevated due to an increased formation of CSF. In meningococcal and other pyogenic meningitis glucose is markedly decreased and may even reach zero level due to its consumption by the infecting organisms and also the large number of polymorphs in the fluid. A reduction of glucose occurs also in tuberculous meningitis though the fluid remains clear and does not contain many polymorphs.

Chloride: The chloride concentration is higher in the CSF than in the plasma. It is lowered in all cases of meningitis, but more so in tuberculous meningitis. In fact, a clear CSF with slight increase in lymphocytes and protein, but marked decrease in glucose and chloride is diagnostic of tuberculous meningitis. The exact cause for the marked fall of chloride in this condition is not known.

Other constituents of CSF do not show variations of any diagnostic significance.

LYMPH

The interstitial fluid is collected by lymph capillaries which empty ultimately into the thoracic duct and right lymphatic duct which join the left and right subclavian veins and thus enters the systemic circulation. The daily lymph flow appears to be 1 to 2 litres in the human adult. Diffusible non-electrolytes like urea and glucose have the same concentration as in plasma. Diffusible non-electrolytes like K^+, Na^+, Cl^- and HCO_3^- tend to be somewhat higher than in plasma. This is on account of the lower protein content of lymph which creates Donnan effect. The protein content of lymph varies with the location from which lymph is collected. It is highest in lymphatics of liver (about 6.0gm%) and lowest in subcutaneous lymphatics (0.25gm%). It averages about 3% in thoracic duct. The albumin/globulin ratio is much higher than in plasma.

Sweat

Its main function is to maintain body temperature. Invisible perspiration accounts for about 600-700 ml/ day and consists of almost pure water. Visible perspiration in hot and humid conditions may contain much of Na^+ and Cl^-. Lactic acid is also present in substantial amounts.

Tears

Tears are secreted by the lacrimal glands and contain 0.6 - 0.8 grams protein per 100 ml. besides electrolytes and other nonprotein nitrogenous materials. The pH is between 7.0 to 7.4 A thin film of watery secretion on the cornea improves vision by covering up microscopic

irregularities on the corneal surface. Tears also contain the enzyme lysozyme which can hydrolyze the glycosidic bonds of mucoproteins in the cell walls of microorganisms. (Lysozyme is also present in salivary secretion, nasal secretion, spleen and leukocytes).

Synovial Fluid

It is secreted by the synovial membrane. Protein content is about 1%. albumin, globulins and proteoglycans are present. Fibrinogen is not present. It is a highly viscous fluid on account of the hyaluronic acid which is present.

Aqueous Humor

It fills the anterior chamber of the eye and maintains the intraoccular tension. It supplies nourishment to the cornea and the lens, both of which are avascular. Its volume is just about 0.25 ml in the human eye. Protein concentration is very low - 0.05 g%. Other substances have similar concentration as in plasma, but the ascorbic acid content is 20 times that of plasma. It is secreted by the *ciliary body*. Any obstruction to its circulation will raise the intraoccular tension and will cause a serious condition called '*glaucoma*'.

Vitreous Humor

This fills the posterior chamber of the eye and consists of a gel of hyaluronic acid in a framework of collagen fibres. Nutrients are exchanged from the blood vessels of the retina and also the aqueous humor of the anterior chamber.

References:

Albritton, E.C.(eds): Standard Values in Blood. Saunders, Philadelphia, 1952.
Antonini, E: Interrelationship between Structure and Function in Hemoglobin and Myoglobin. Physiol. Rev., 45:123, 1965.
Biggs, R. and McFarlane, R.G.: Human Blood Coagulation and its Disorders, 3rd., ed., Davis, Philadelphia, 1962.
Bishop, C. and Surgenor, D.M. (eds): The Red Blood Cell., Acad. Pr., N.Y., 1964.
Cinader, R.(ed): Regulation of the Antibody Response. C. Thomas, Springfield, Ill., 1967.
Ingram, V.M.: The hemoglobins in Genetics and Evolution., Columbia Univ. Pr., N.Y., 1963.
Janeway, C.A. et.al: The Gamma Globulins., Little Brown, Boston, 1967.
Putnam, F.W. (ed.): The plasma Proteins, Vol. 1 and 2, Acad, Pr., N.Y., 1960.
Schroeder, W.A.: Hemoglobins., Ann., Rev, Biochem., 32:301, 1963.

N-Acetylmuramic acid is formed by esterifying the -OH of the C-3 of N-acetyl-glucosamine with D-lactic acid. The -COOH of lactic acid combines with a tetrapeptide consisting of L-alanine, D-isoglutamic acid, L-lysine and D-alanine. The second and third amino acids are variable and may be replaced by D-glutamate or glutamine and by hydroxylysine, ornithine or mesodiaminopimelic acid. They vary from species to species. The polysaccharide chains are interlinked by the peptide chains. The peptide chains may themselves be cross linked by short connecting peptides like pentaglycine.

The peptidoglycan structure resists digestion by proteolytic enzymes which do not act on peptide linkages involving D-amino acids. But the enzyme 'LYSOZYME' (present in tears) can hydrolyze the β 1-4 glycosidic bonds of the bacterial polysaccharide of the cell walls of Gram-positive bacteria. The cell wall weakens and the cell swells up and ruptures. If the lysozyme is allowed to act in high concentrations (0.8M) of sucrose medium, sucrose being impermeable to the cell membrane, does not produce osmotic swelling of the cell. The naked bacterial cell surrounded by its membrane (but without the cell wall) is called s 'PROTOPLAST'.

In addition to the peptidoglycan, the cell walls also contain certain other polymers– 1. Teichoic acids, 2. Polysaccharides and 3. Polypeptides. In Gram-positive bacteria, teichoic acids are polymers of glycerol or ribitol linked togther by phosphodiester bridges. Ribitol may be substituted by D-alanine, D-glucose or N-acetyl, D-glucosamine. The polysaccharides are made up of rhamnose, glucose, galactose or mannose or their amines. Teichoic acids and polysaccharides exibit antigenic properties.

In Gram-negative bacteria, like E. coli, the necessary components consist of polypeptides, lipoproteins and a highly complex lipopolysaccharide. The repeating unit of the lipopolysaccharide is a trisaccharide consisting of two heptose (7-carbon) sugars and an octulosonic acid (8-carbon sugar acid). These polysaccharides are linked together by small oligosaccharides. The fatty acid is β - bydroxy myristic acid (14 carbons). The antigenic specificity of Gram-negative bacteria is on account of this lipopolysaccharide.

Blood group proteins contain oligosaccharide side chains of L-fucose, D-galactose, N-acetyl, D-galactosamine and N-acetyl-D-glucosamine. The specificity of the blood groups is determined by their side chains.

Cell membrane: This is the outermost covering of the animal cell and is responsible for the size and shape of the cell, both of which can however be readily altered if necessity arises. It is not a rigid structure.

The cell membrane is as much a living structure as the rest of the cell constituents and undergoes constant renewal. It is made up mainly of lipid and protein. The lipid is mainly phospholipid - lecithin and cephalin.

The cell membrane shows many invaginations which go into the cell and even encircle the nucleus. The inner membranes like the endoplasmic reticulum and mitochondria also have similar gross structure as cell membrane.

Current concepts of membrane structure are based on the fluid mosaic model proposed by Singer and Nicolson (1972). The lipids (phospholipids, glycolipids and cholesterol) form bimolecular sheets, the hydrophilic polar regions (glycerol and sphingol) forming the outer and inner surfaces while the hydrophobic, non-polar portions (fatty acid chains) form the inner core (in two layers) of the membrane. The bimolecular sheet is highly impermeable to ions and most polar molecules. It is quite fluid in nature and acts as a solvent for the membrane proteins. The proteins are mostly large, globular proteins, some of which penetrate the matrix from the outer surface to the inner surface. They are called *"tunnel proteins"* and are important for the transport of substances through the membrane. Some smaller protein molecules do not penetrate the matrix, but lie embedded in the outer or inner surface. In either case, the hydrophilic (polar) regions of the protein are exposed on the outer or inner membrane surface and the hydrophobic (non-polar) regions are in contact with the lipid matrix. (see fig. 7.2)

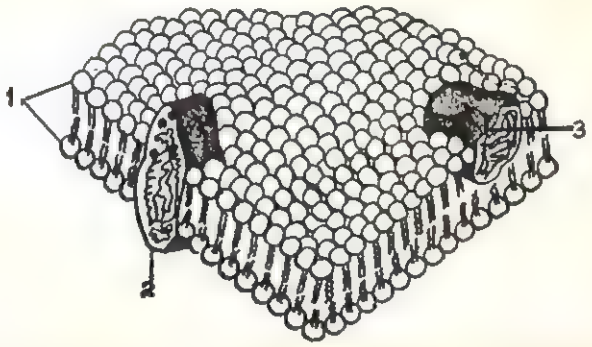


Fig. 7-2 Structure of cell membrane (fluid mosaic model)

1. Polar groups of phospholipid
2. Tunnel protein (or integral protein)
3. Peripheral protein

The membranes also contain small amounts (2-10%) of carbohydrate in the form of glycolipid (cerebrosides) mainly.

Membranes contain specific receptors for external stimuli eg. for insulin, light etc. They are also capable of generating signals - electrical or chemical. A large number of enzymes are located in the membranes. Protein - lipid ratio of the membrane varies from 1:4 to 4:1 in different membranes. Protein content is high in enzyme-rich membranes like the mitochondrial membrane. Lipid content is high in the myelin sheathes covering nerve fibres. Here the membrane acts as an insulator.

The carbohydrate component of the membrane is responsible for intercellular recognition and plays a role in the antigen - antibody recognition and response.

Nucleus: The nucleus consists of one or more small, round, darkly stained bodies inside the cytoplasm, lined by a nuclear membrane. Electron microscopy reveals occasional channels connecting the cell membrane to nuclear membrane. It is possible that the nuclear membrane might have arisen as an invagination of the cell membrane. The nucleus is filled with a cytoplasm-like material called nucleoplasm. In interphase, i.e. the period between mitoses, the eukaryotic nucleus contains an irregular network of chromatin, staining deeply with basic dyes. The chromsomes are not demarcated. They become visible only during mitosis. They contain DNA combined with basic proteins called histones which are low molecular weight (10,000 to 20,000) basic proteins. The DNA is thrown into several loops and folds. Human cell contains 46 chromsomes.

The nucleolus is a small body within the nucleus and contains mostly RNA protein.

Mitochondria: These are 0.5 to 7.0 μ long) and 0.5 μ thick. Several hundreds to a few thousands are present in the cytoplasm of each cell. They contain protein, triglyceride, phospholipids and nucleic acid - mainly RNA.

Mitochondrial DNA is double stranded and circular and much smaller than chromosomal DNA. It is only about one hundredth of what is present in the nucleus.

The mitochondrial membrane also is a double layered structure. The inner membrane is 60 - 80 Å thick and is thrown into deep folds into the inside of the sausage like structure. The folds are called cristae and help in increasing the surface area of the membrane and also in subdividing the whole structure into several intercommunicating chambers. The structure is shown schematically in Fig. 7-3.

Fig. 7-3. Mitochondrion (Schematic)

In some tissues, they are especially located at the site of maximum energy requirement *eg.*, contractile fibres of muscle cells and around the midpiece of the spermatozoan. The number per cell also vary with energy requirements - 250 per sperm cell and 500 - 2,000 in liver cell. A fifth of the protein in liver cell is on account of mitochondria.

The cristae are more numerous and tightly packed in tissues like the heart muscle which have active oxidative mechanism. They are loosely packed in liver cells in which the metabolic reactions are numerous, but not mainly oxidative.

The cristae offer a large surface area. The inner membrane of the cristae is studded with innumerable projections called 'elementary bodies' each of which consists of a 'head piece' connected through a 'stalk' to a 'base-piece' located in the inner membrane, (see Fig. 7-4). The head piece contains ATP-ase activity. The base piece contains the electron transport system. In between the outer limiting membrane and the inner membrane is the soluble phase. This contains the enzymes like phosphatases, kinases, urea cycle enzymes and also cytochrome-C. In the space enclosed by the inner membrane (into which the elementary bodies project) is the 'matrix' which contains the enzymes of the citric acid cycle and beta oxidation. The membranes themselves can be separated from one another by subjecting the mitochondria to alternate exposure to hypotonic and isotonic saline. The thin inner membrane ruptures and can be separated from the more dense outer membrane. The outer membrane contains all the monoamine oxidase activity of the mitochondrion.

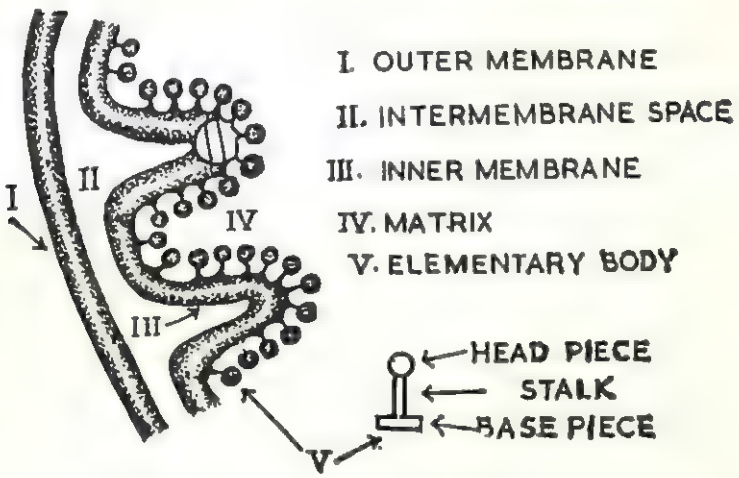


Fig. 7-4 Detailed structure of cristae

Microsomes: These are particles 100 to 150 Å in diameter, visible under electron microscope, attached on a network of thin membrane-like tubules and vesicles. The network itself is called the 'endoplasmic reticulum . Some particles may also occur free in the cytoplasm. The particles are made up mainly of RNA protein and are also called 'ribosomes'. They are the active sites of protein synthesis within the cell.

Golgi apparatus: This is discernible when selective stains like osmium tetraoxide or silver salt are used. It is a complex consisting of membranes and vacuoles with smooth surfaces (X endoplasmic reticulum). It is richer in lipid than endoplasmic reticulum. It is considered to be the site where the secretions from other cell structures (organelles) are brought and assembled

Lysosomes: They are intermediary in size between mitochondira and microsomes (0.25 to 0.8 μ diameter). They are sacs containing hydrolytic enzymes which will be released into the cell on rupture of the membrane.

Chloroplasts: They are present only in plant cells and contain the green pigment chlorophyll besides protein (56%) and lipid (32%). The lipids contain carotene and xanthophyll derivatives and also triglycerides, phospholipids and waxes. The chloroplast is a lamellar structure and dense green bodies called grana (0.3 - 1.7 μ long) are the active sites of photosynthesis.

The membrane of chloroplast has similar properties as the mitochondrial membrane including the presence of small amounts of DNA. They can probably replicate themselves independent of the chromosomal DNA of the nucleus.

Cytoplasm: The structureless material filling the rest of the cell is a colloidal solution of proteins containing organic substances like glucose and inorganic salts of potassium, magnesium and the like and is termed the cytoplasm. Small amounts of RNA and enzymes of glycolytic pathway are also present in the cytoplasm.

Cell Motility and the Cytoskeleton

Cells other than the muscle cells also show some amount of mobility in size, shape and position. This is possible on account of an extensive intracellular network of filamentous structures which is given the name '*cytoskeleton*'. Three types of filamentous structures are observed in the eukaryotic cells -
i. actin filaments, ii. microtubules and iii. intermediate filaments.

The actin filaments are 0.7-9.5 nm in diameter and, just like in muscle, can polymerize to form F-actin. The microfilaments so formed form a meshwork in the cytoplasm. In structures like the microvilli of the intestinal mucosa, these actin microfilaments are arranged along the length of the microvillus. At the base, myosin filaments occur. By an interaction between the actin and myosin (a sliding action), movement of the villus is brought about. Tropomyocin and several other protein factors are also found to occur in cells other than muscle.

Microtubules: These have a diameter of about 25 nm. and extend to variable lengths in the cell. They facilitate movement of the vesicles and also help in the formation of the mitotic spindle (during cell division) and in the formation of cilia and flagella.

Intermediate filaments: They have diameters from 10-12 nm. They are made up of different proteins like keratin, desmine etc. and form stable components of the cytoskeleton.

The Cell Cycle

Different cells of the eukaryotes show great variations in their life cycle. Mouse hepatoma cells grown in culture media manifest four different stages –
1. An 8 - 10 hour period of pre-DNA synthesis, during which there is synthesis of RNA and protein.
2. Another 8 - 10 hour period of active DNA synthesis. The RNA and protein synthesis continue in this phase also.
3. A three to four hour period of post DNA synthesis. The RNA and protein synthesis continue during this phage.

4. A one hour period of mitosis during which the chromosomes separate. All synthetic processes stop during this phage. The cell divides into two and each daughter cell goes through the cycle of four stages again.

The entire cycle lasts 18-24 hours and is called the *cell cycle.*

Mammalian nerve cells do not undergo cell division in the adult and do not synthesize DNA. But they do synthesize RNA and protein and survive for the life time of the individual. Mature lymphoid cells also do not divide.

Mammalian erythrocytes lack DNA and the ability to synthesize any new protein. They have a fixed life span of about 120 days in man.

COMPOSITION AND METABOLISM OF SPECIALIZED TISSUES

Muscle

Muscle is the largest single tissue in the human body and contributes 25-40% of body weight. Its main function is to convert chemical energy released by the breakdown of ATP to mechanical energy in the form of muscle contraction. The basic contractile unit of the muscle is a *sarcomere* which is made up of interdigitating filaments — thick filaments made of *myosin* and thin filaments made of *actin*. On stimulation by a nerve impulse, and with the energy supplied by the breakdown of ATP, the thin filaments slide past deeper into the array of the thick filaments, thus effectively reducing the length of the sarcomere. The entire muscle is made up of a number of longitudinal fibres called muscle fibres. Each muscle fibre, in turn, is made up of smaller fibres called myofibrils. Myofibrils are formed by a number of sarcomeres arranged end to end. The result of a coordinated action of a stimulus on all the sarcomeres is therefore to shorten the length of the muscle — muscular contraction. It may be however noted that the contraction or shortening is produced *not* by shortening of the contractile elements — the myosin and actin filaments — but by their moving deeper into the space around each other. A detailed description of the components involved follows.

Myofibrils: (See Fig. 7-5). These are the contractile units in striated muscle and are of 0.5-2.0μ diameter. In skeletal muscle, the fibrils also show cross striations — wide bands called A bands alternating with narrower bands called the I bands. The A bands are rich in the fibrous protein myosin and are strongly birefringent (anisotropic; hence called 'A bands'). What are described as a H disc and an M line can also be distinguished in the A band. The I band is isotropic (hence called I band) and shows two lines — the N & Z lines.

The A band is said to be made up of filaments 10nm diameter. As they enter the H region in the band, they thicken to 14nm, thus giving the appearance of a disc to that region. The I band contains only filaments of uniform diameter of 6nm which also enter from the I band into the A band on either side up to the H disc. So there is an overlap of the A & I filaments in the A band, while only I filaments are present in the I band.

Sarcomere is the name given to the region between the centre of one I band to that of the next I band (includes a complete A band in the middle, with half of I band on either side)

and is considered as a single functional segment. In the rabbit skeletal muscle, this is 2.5μ long in the relaxed state, the I band (2 halves) accounting for 1μ and the A band 1.5μ

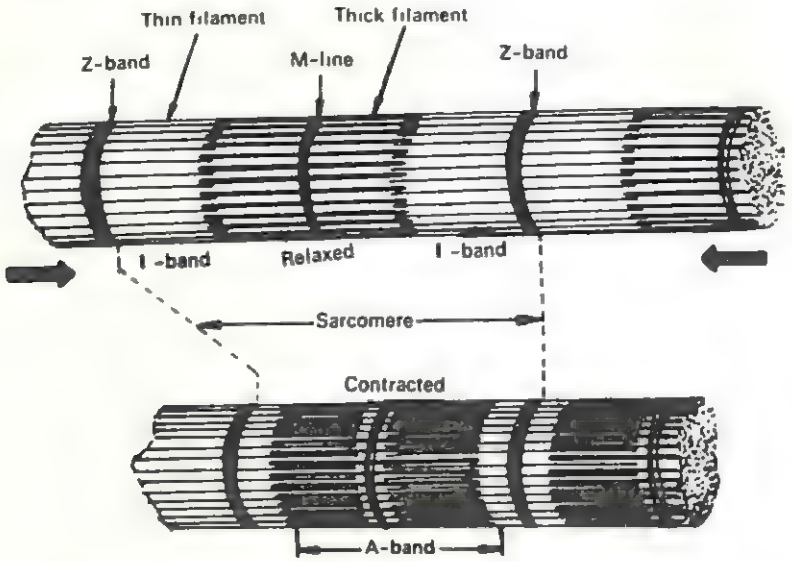


Fig. 7-5. Structure of striated muscle

The structure is shown in the relaxed state above and the contracted state below. In the relaxed state, note that the thick filaments in the A-band and the thin filaments in the I-band just interdigitate. The Z-bands form the limits on either side to a sarcomere and anchor the thin filaments.

In the contracted state, the thin filaments enter the A-band deeper, thus pulling the Z-bands together. The length of the sarcomere thus becomes shorter. Please note that there is no shortening of the A-band (thick filaments). There is a shortening of the I-band. But this is not due to any shortening of the thin filaments, but due to their deeper penetration into the A-band.

The cross striations are not present in smooth muscle. The muscle filaments are also not strictly parallel. Cardiac muscle shows striation, but the individual cell differentiation is non-existent. Instead, a network or syncitium is formed. Smooth muscle exhibits spontaneous activity. Cardiac muscle also exhibits spontaneous activity but to a much less extent. Among the skeletal muscle fibres, some fibres are red, others white. Red fibres are less responsive to stimuli and get into fatigue late. White fibres are more responsive and more easily fatiguable. The red fibres contain more myoglobin, cytochromes, more number of mitochondria and larger sized mitochondria than the white fibres. The white fibres, on the other hand, contain less of ATP and anserine. The red fibre derives its energy mainly by oxidative reactions while the white fibre mainly by glycolysis. The anserine is probably useful in neutralizing the acid products of glycolysis.

Sarcolemma: It covers the muscle fibres and consists of two layers — one outer layer of reticular fibres and an inner membranous layer. The inner membranous layer is attached to the muscle fibre at the Z line, possibly at the M line also–the middle of the H-Zone. The sarcolemma is in a polarized state (100 mv + ve, outer surface) and becomes depolarized during muscle contraction. Permeability properties of muscle are on account of the sarcolemma.

Sarcoplasmic reticulum: The membrane excitation is said to be transmitted inwardly to the fibrils through the sarcoplasmic reticulum. Some components of the reticulum are also said to act as the 'relaxing factor'

Proteins of sarcoplasm: Sarcoplasm is a colloidal solution of a complex mixture of proteins and bathes all the constituents of the muscle cell. The proteins are readily soluble in buffers of low ionic strength (while the proteins of myofibrils are insoluble). They are albumin-like and are called myogens A & B. There is also a globulin-like protein called globulin-X. Each is a mixture of several proteins. It contains the glycolytic and other enzymes. The myofibrils are bathed in the sarcoplasm without any membrane intervening.

Microsomes are present in the sarcoplasm and they are responsible for synthesis of the muscle proteins.

Proteins of Myofibrils: Myosin, actin and tropomyosin are the main proteins. The actin and myosin are associated as actomyosin.

The thick filament

The thick filaments are made up of myosin molecules. Each myosin molecule consists of 6 polypeptide chains. Two of them form the entire shaft (156 x 2 or 3 nm) and a part of the two globular heads at the NH_2 end. The shaft consists of the alfa helical portion of the two polypeptide chains forming a compound coil. A pair each of light chains add on to the two globular heads to complete the structure. About 400 of myosin molecules are stacked to form one thick filament. The heads are placed in a spiral fashion along the length of the filament. The tail ends meet in the mid-zone (at the M-line) which does not contain any heads. These shafts are held together by a special protein called the M-protein.

The thin filament

The thin filaments are made up of a double stranded chain of beads. Each bead represents a G-actin molecule. The two helical chains twisting around the beads are tropomyosin molecules. Troponin molecules, each consisting of 3 subunits, occur at regular intervals along the length of the thin filament.

Interaction between the thick and thin filaments:

In the relaxed state, the sarcoplasm has a high concentration of ATP and Mg^{++}. The concentration of Ca^{++} is below the threshold level. There are no cross linkages between the thick and the thin filaments. Each myosin head contains 2 molecules of ATP in a tightly bound form. The tropomyosin molecules mask the myosin binding sites on G-actin. Free Ca^{++} is now released into sarcoplasm by the incoming nerve impulse. The Ca^{++} is immediately taken up at the calcium binding site on troponin which now undergoes conformational change resulting in exposing the myosin binding site of G-actin. The G-actin will combine with the ATP bound myosin head and forms the force generating complex.

The myosin head now undergoes a conformational change with respect to the axis of the heavy filament which results in causing the thin filament to slide along the thick filament and the bound ATP is released as ADP and phosphate.

Relaxation is brought about when the Ca^{++} moves back from the sarcoplasm into the sarcoplasmic reticulum.

Stimulus for muscle contraction: The stimulus for muscular contraction is a nerve impulse. It reaches the motor end plate or the neuromuscular junction and rapidly spreads over the sarcolemma.

In the resting muscle, there is a potential difference across the sarcolemma, the outer surface being electropositive by about 60 millivolts compared to the inner surface. As the excitation impulse spreads over the sarcolemma, this potential difference between the two surfaces disappears. The membrane is *depolarized*. This is due to a sudden increase in the permeability of the membrane to K^+, Na^+ and Ca^{++} which flow in such a direction as to result in discharging the transmembrane potential. The sarcolemma forms innumerable tubular invaginations which run across the muscle cell near the Z-lines or the A-I junctions. This is called the *T-system* and helps in transmitting the wave of excitation from the nerve impulse simultaneously to all the sarcomeres in the muscle fibre.

The wave of excitation and depolarization in the sarcolemma is transmitted to the closely apposed membranes of the sarcoplasmic reticulum which now becomes permeable to Ca^{++} ions. The calcium ions pass out into the sarcoplasm and trigger the changes leading to contraction of the myofibrils already described. Calcium ions remain in the sarcoplasm as long as nerve impulses continue to reach the sarcolemma and keep the muscle fibres in the contracted state.

When nerve impulses cease, the sarcolemma regains its original permeability pattern and attains once again the polarized state. The membrane of the sarcoplasmic reticulum likewise returns to the original state and calcium ions are actively transported back into the reticulum from the sarcoplasm. This requires energy from the breakdown of ATP to ADP and is described as the calcium pump. Special calcium binding proteins are said to exist in the membrane of the sarcoplasmic reticulum which help in segregation of calcium by that structure in the relaxed state.

Thus muscle contraction as well as muscle relaxation require energy from ATP. The concepts described above are shown schematically in Fig. 7-5 and 7-6.

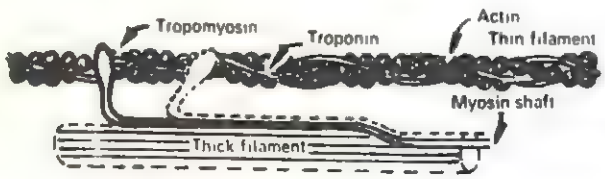


Fig. 7-6. A schematic representation of muscle proteins and their interaction during muscle contraction

The continuous line indicates the myosin molecule in the relaxed muscle. The myosin head is in loose contact with the actin in the thin filament.

The broken line indicates the myosin molecule in the contracted muscle. The myosin head gets bound to the actin of the thin filament and bends, pulling the thin filament into the A-band.

Other constituents: Nucleotides like ATP, ADP, ITP, GTP, UTP and their derivatives, enzymes like L-myosin ATP-ase, myokinase, deaminases etc., are also present. ATP is produced by reactions of glycolysis, citric acid cycle and β-oxidation of fatty acid as in general metabolism. Excess ATP not required for immediate use is used for converting creatine to creatine ~ P.

$$\text{Creatine} + \text{ATP} \xrightarrow[\text{(creative phosphokinase)}]{} \text{Creatine} \sim \text{P} + \text{ADP}.$$

Rigor mortis: This is a condition of stiffening of muscles after death. ATP level falls due to increased rate of breakdown of ATP compared to its rate of regeneration. Creatine ~ P level also is decreased. Actin becomes irreversibly combined with mysoin and the actin and myosin fibrils get irreversibly locked together at the actin interacting sites of the proteins.

Connective tissue

It is present all over the body and fills up the gaps between the several viscera and forms their frame work. The capsules of joints, tendon and muscle sheathes, the dermis of the skin, the supporting tissue around the blood vessels, nerves and lymphatics are all examples of connective tissue.

It consists of (1) ground substance (2) fibres and (3) cells.

1. Ground substance: It is a gel like substance containing water, salt, proteins and polysaccharides. It fills up the extracellular space and has a slimy feel. The polysaccharides are also described as muco-polysaccharides. Hyaluronic acid, chondroitin sulphate and heparin are a few examples. They are complex polysaccharides usually containing glucosamine or galactosamine and glucuronate units. They are united to eah other by 1-4 or 1-3 glycosidic linkages. The amino group is usually acetylated or sulfated. Due to the ionization of these acid groups, they are strongly anionic and combine with cationic proteins to form muco-proteins (glycoproteins). The mucoproteins have very large molecules and very high molecular weights.

The enzyme, hyaluronidase, which is present in some micro-organisms, snake venom and seminal fluid, hydrolyzes the polysaccharide hyaluronic acid and thus makes the ground substance readily permeable to the micro-organisms or their toxins. Infection thus spreads in the tissue. Hence it is called the 'spreading factor'. In case of seminal fluid, it helps in clearing the way for the spermatozoa to reach the ovum.

Adrenal cortical hormones and growth hormone regulate the synthesis of the muco-polysaccharides. Vitamin C deficiency and diabetes mellitus decrease their synthesis. Hence delayed wound healing occurs in these two conditions.

2. Fibres: Three types of fibres are present — collagen, elastin and reticulin.

(a) Collagen: This forms the largest part of the fibres. It is a component of most connective tissues including bone, and is responsible for the tensile strength of the tissues. About 30% of all protein in the body is collagen. It is a fibrous, elongated molecule, 2900 Å x 15 Å. It is synthesized by fibroblasts and other mesenchymal cells, but, finally becomes extracellular. The aged fibres gradually become less soluble, tougher and less elastic.

It contains a large preponderance of three amino acids — glycine, proline and hyroxyproline, the last named occurring only in collagen. Hydroxylysine is also present in small amounts. Every third amino acid inthe polypeptide chain is glycine; every fifth amino acid is proline or hydroxyproline. The presence of ring structures of proline and its hydroxy derivative in such abundance makes it difficult for a true alfa helical structure to be formed. But the polypeptide shows a general left handed turn in such a way as to bring the short glycine residues on to one surface and the ring structures to another. Three such peptide chains are intertwined with a right handed twist so as to bring closely together the glycine-bearing surfaces to form a compact rope-like structure (see Fig. 7-7).

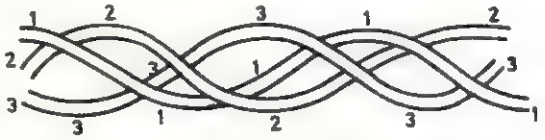


Fig. 7-7. Structure of collagen

Esterlike bonds between carboxylic groups of aspartic acid residues with OH groups of neighbouring chains will hold the chains firmly together and will prevent their sliding over one another. Aldehyde groups formed by oxidation of lysine residues or by reduction of c-terminal COOH groups will condense with a lysine residue of the neighbouring peptide chain and will further help in firmly binding the chains together.

The lysine-aldehyde binding is prevented by an agent (beta-aminopropionitril) present in a species of peas of genus Lathyrus. Consumption of these peas in food for long periods will cause a disease involving the connective tissues and the nervous system and is called 'lathyrism'

Deficiency of vitamin C results in a disease called scurvy, a disease mainly involving connective tissue. The hydroxylation of proline to hydroxyproline requires vitamin C.

Inherited Disorders of Collagen

Ehler-Danlos Disease: Several types are described. There may be a defect in the normal synthesis of collagen, in the hydroxylation of lysine, in the conversion of procollagen to collagen etc. The disease is manifested by fragility of skin, hyperextensibility and hyperflexibility of joints and skeletal deformities. Most of the cases are due to defect in hydroxylation of lysine.

Osteogenesis Imperfecta Congenita: Increased fragility of bones and pathological fractures are common in this. The defect is in the formation of normal procollagen.

Cutis Laxa: Skin is lax and joints hypermobile. There is a deficiency in the hydroxylation of lysine.

Marfan's Syndrome: Skeletal deformities, cardiac disease, aortic aneuyrism, long and thin digits and dislocation of the lens are some of the manifestations of this condition. The cause is not known.

(b) Elastin: Elastic tissue is yellow and refractive and has a fibrillar, membranous or intermediate structure. It is seen in large arteries, skin, lungs and ligaments. It is a scleroprotein and is insoluble in most neutral solvents. Glycine, alanine, valine, proline and leucine are the chief amino acids present. Glycine occurs in high concentrations as in collagen. The fibre has an elastic rubber like consistency. Some hitherto not described amino acids which are tetra-carboxylic and tetra-amino acids were recently isolated from elastin (desmosine and isodesmosine).

(c) Reticulin: They are thin collagen fibres which precede the thicker ones. There is another type of reticulin which is associated with basement membranes, nerve and muscle fibres. It is a complex of collagen, lipid and polysaccharide.

Some special type of proteins also occur in some tissues.

(i) Fibronectin: This is a glycoprotein present on the cell surfaces, extracellular matrix and in blood. Proteoglycans-heparan sulfate and chondroitin sulfate are also associated with the matrix.

(ii) Keratins: These are hard, tough proteins present in the epidermal structures like nail, horn, hoof and skin. They consist of fibres of alfa keratin in a matrix called *keratohyaline.* The keratin fibres consist of three polypeptide chains like collagen. The matrix is rich in basic amino acids in the epidermis of the skin and in the sulfur containing amino acid, cystine, in the nail, horn and hoof.

(iii) Tubulin: The microtubules present in the mitotic spindle, the cytoskeleton of most eukaryotic cells, the flagella, the spermatozoa and the neurotubules contain tubulin, a filament like protein. The filaments are arranged in such a way as to form a tubule between them.

3. Cells: They consist of fibroblasts, osteocytes, chondrocytes, mast cells and reiculoendothelial cells and occur depending on the nature of the tissue.

Nerve tissue

This constitutes about 2.4% of the body weight of man.

Brain	1400 gms
Spinal nerves	150 "
Spinal cord	30 "
Cranial nerves	10 "
	1590 or 1600 gms

The chemistry of the nerve tissue is well known but the chemistry of its functional aspects is little understood. Nerve tissues are rich in lipids.

	Total lipid (% of dry weight)
Gray matter	38
White matter	64
Myelin	80
C.S.F.	Traces
Plasma	0.5

A little over half (51-54%) of the solids of nerve tissue are lipids. Phospholipids account for more than half of the lipids, cholesterol forms about 20%, cerebrosides 14% and other lipids (sulpho and amino lipids) about 18% of the lipids. The phospholipids are mainly cephalins, lecithins and sphingomyelins. All the cholesterol is in the unesterified form.

The myelin sheath is made up of a series of layers of the external membrane of the Schwann cell around the axon. The membrane is double layered (protein-lipid, lipid-protein).

Proteins constitute about 40% of the dry weight of nerve tissue (8% of fresh brain). Proteins that can be classified as albumins, α_1, α_2, β_1, β_2 and γ globulins and also fibrinogen were all isolated from brain proteins. However the albumin forms a minute fraction (2% compared to 55% in plasma proteins) while the β globulins form the major fraction (65% compared to 13% in plasma proteins): The other fractions do not show so much variation from plasma proteins.

A special protein in brain containing two atoms of copper per molecule with a molecular weight of 35,000 is called 'cerebrocuprein I'. This is increased in Wilson's disease. There is a corresponding decrease of the copper containing protein in plasma called 'ceruloplasmin' and an increase in the plasma levels of free copper.

Brain contains about 6 times as much free amino acids as plasma. It is particularly rich in aspartic and glutamic acids and their derivatives (N-acetyl aspartic acid, glutamine and γ - amino butyric acid). Taurine and glutathione also are present in fair amounts.

Blood-Brain Barrier and Blood-Cerebrospinal Fluid Barrier: The blood capillaries are surrounded by an extra layer of glial cells and they limit the passage of many charged organic and inorganic molecules and large molecules like lipid, polypeptides and polysaccharides. Hence the brain metabolism differs in some respects from other tissues.

Metabolism of brain: This accounts for 25% of O_2 utilized by the body at rest. The respiratory quotient of brain is close to unity which indicates carbohydrate as the main substrate for the brain metabolism. Very little carbohydrate is stored in brain (0.1% is the glycogen content of brain). Hence there must be a continuous supply of glucose from blood to maintain normal function of this vital tissue. Glucose is readily pemeable through the blood brain barrier. A decrease in O_2 supply or glucose supply will markedly decrease metabolism and ATP and creatine phosphate levels of brain. During sleep and anaesthesia, there is an increase in ATP and creatine phosphate due to lowered activity of the brain. In convulsions, spontaneous or induced by drugs, there is rapid depletion of these energy rich phosphates; inorganic phosphate and lactic acid accumulate.

Glycolysis and citric acid cycle seem to be the main pathways of metabolism in brain.

In the absence of adequate carbohydrate, the citric acid cycle is maintained by converting glutamic acid to α -ketoglutarate, an intermediate in the cycle. Glutamic and aspartic acids also play an important role in the removal of ammonia.

$$\text{Glutamic acid} + NH_3 \xrightarrow{\text{ATP}} \text{Glutamine}$$

$$\text{Aspartic acid} + NH_3 \xrightarrow{\text{ATP}} \text{Asparginie}$$

Glutamine is readily permeable through blood-brain barrier and enters the circulation. In the kidney, the reaction is reversed in the renal tubular epithelium and the ammonia is excreted in exchange for Na^+ and K^+ ion.

Gamma amino butyric acid formed as a result of decarboxylation of glutamic acid is said to be a synaptic transmitter for inhibitory neurons.

Serotonin (5-hydroxytryptamine) formed from tryptophan is an excitor. It is produced within the brain itself and is not permeable to blood-brain barrier. Hence serotonin produced in the blood platelets or gastrointestinal tract has no cerebral effect. Reserpine — an antihypertensive and sedative drug — acts by releasing the serotonin from protein combination and facilitating its rapid destruction by the enzyme monoamine oxidase.

Lysegic acid diethylamide (LSD) causes excitation and hallucinations by inhibitng monoamine oxidase and thus prolonging serotonin action.

Conduction of the Nerve Impulse

The nervous system is a network of living fibres which interconnect with each other and also with other cell types like sensory receptors, muscle, secretory cells etc. The junction point is called a *'synapse'*. The receptor and the nerve ending are not directly connected. There is a small gap, about 2-20nm. If the gap is very small (about 2nm), the nerve impulse may directly flow by jumping the gap. But in most cases, it requires to be transmitted by chemical means. A transmitter substance is released from the presynaptic membrane, crosses the gap by diffusion and binds to a specific receptor on the postsynaptic membrane. *Acetylcholine* is the synaptic transmitter for many of the neurons.

The axons are cylindrical fibres capable of conducting electrical impulses. Their plasma membrane is about 6nm thick and is non-conducting. In the resting condition it is in a highly polarized state, 75mv negative inside. This is because K^+ is 20-50 times more concentrated in the axon and Na^+ is only about 1/20th of plasma. Na^+, K^+ and Cl^- are permeable through the membrane, but in the resting state, they do not show much movement in either direction. The principal anions inside — proteins and nucleic acids are impermeable. To maintain this differential concentration, the sodium pump operates. Na^+, K^+ –ATPase on the axon membrane pumps out 3 Na^+ for every 2 K^+ which enter the cell. One ATP molecule is broken during the process.

The Action Potential

Any stimulus which can cause a depolarization and reduce the electric potential at a point to below –50mv (from the initial –75mv) will trigger a series of events which will result in an

'action potential'. The permeability of the membrane to Na^- greatly increases and Na^+ rushes into the cell due to its higher concentration in the plasma. It may actually cause an overshoot so that the electric charge on the inside of the membrane may become + 30mv. This positive charge repels further entry of Na^+ into the cell, the permeability of the membrane to Na^+ falls rapidly and the Na^+, K^+ –ATPase is stimulated to pump out Na^+ to restore the electric potential to the base level. Special channels for sodium ion are said to be present in the membrane. These channels can be blocked by poisons like 'tetrodoxin' (from a type of fish) and the toxin contained in the scorpion venom. They can therefore block the transmission of nerve impulse.

This transient alteration in the electric potential on the inside of the membrane sets up an 'action potential' which is transmitted along the length of the nerve fibre to the synapse. The duration of the aciton potential is about a millisecond or less and is transmitted at a rate of 1-50 meters/second depending on the thickness and nature (myelinated or not) of the nerve fibre.

Transmission at the Synapse

On the presynaptic membrane are small vesicles called '*synaptosomes*' which contain acetylcholine. Arrival of the action potential causes a release of Ca^{++} ions and an opening of the synaptosome membranes to release acetylcholine. The presence of acetylcholine depolarizes the postsynaptic membrane and causes an action potential to be generated there. The wave of electric potential generated initially thus gets transmitted. The action of acetylcholine is short and is terminated by the breakdown of acetylcholine to acetate and choline by the enzyme '*acetylcholinesterase*' which is present in the synaptic space. Acetylcholine is resynthesized in the synaptosomes by the action of the enzyme '*choline-acetyltransferase*'.

$$\text{Choline + acetyl CoA} \longrightarrow \text{Acetylcholine + CoA}$$

Not only acetylcholine, but a number of other substances also act as neurotransmitter substances, in different components of the nervous system. Epinephrine, norepinephrine, serotonin and dopamine are some examples. Gamma-aminobutyrate and glycine act as inhibitory transmitters by increasing the permeability of the membrane to Cl^- and thereby increasing the negative polarity on the inner side of the membrane. This requires the generation of a larger impulse, in order to get propagated. Many of the lesser impulses are blocked.

The role of endorphins and enkephalins in neurotransmission is discussed elsewhere.

Adipose Tissue: See under 'Lipid Metabolism'.

Bone and Teeth: See under 'Mineral Metabolism'.

Liver: See under 'Assessment of Liver Function'.

References:

Elliot, K.A.C., Page, I.H. and Quastel, J.H.: Neuro-Chemistry, C. Thomas, Springfield, III, 1955.
Kleinzeller, A. and Kotyk, A. (eds): Membrane Transport and Metabolism, Acad. Pr., N.Y., 1961.
Langley, L.L.: Cell Function, 2nd ed. Van Nostrand Reinhold Co., Princeton, N.J. and Affiliated East West Pr. New Delhi, 1969.
McIlwain, H.: Biochemistry and Central Nervous System, 2nd ed., Little Brown, Boston, 1959.
Nachmonson, D.: Chemical and Molecular Basis of Nerve Conduction., Acad. Pr., N.Y., 1959.
Rodhal, K. and Horvath, S.M. (eds): Muscle as a Tissue., McGraw-Hill, N.Y., 1962.
Szent-Gyorgyi, Chemistry of Muscular Contraction, Acad. Pr., N.Y., 1951.
Turnbridge, R.E. (ed): Connective Tissue., Blackwell, Oxford, 1957.

8

ENZYMES

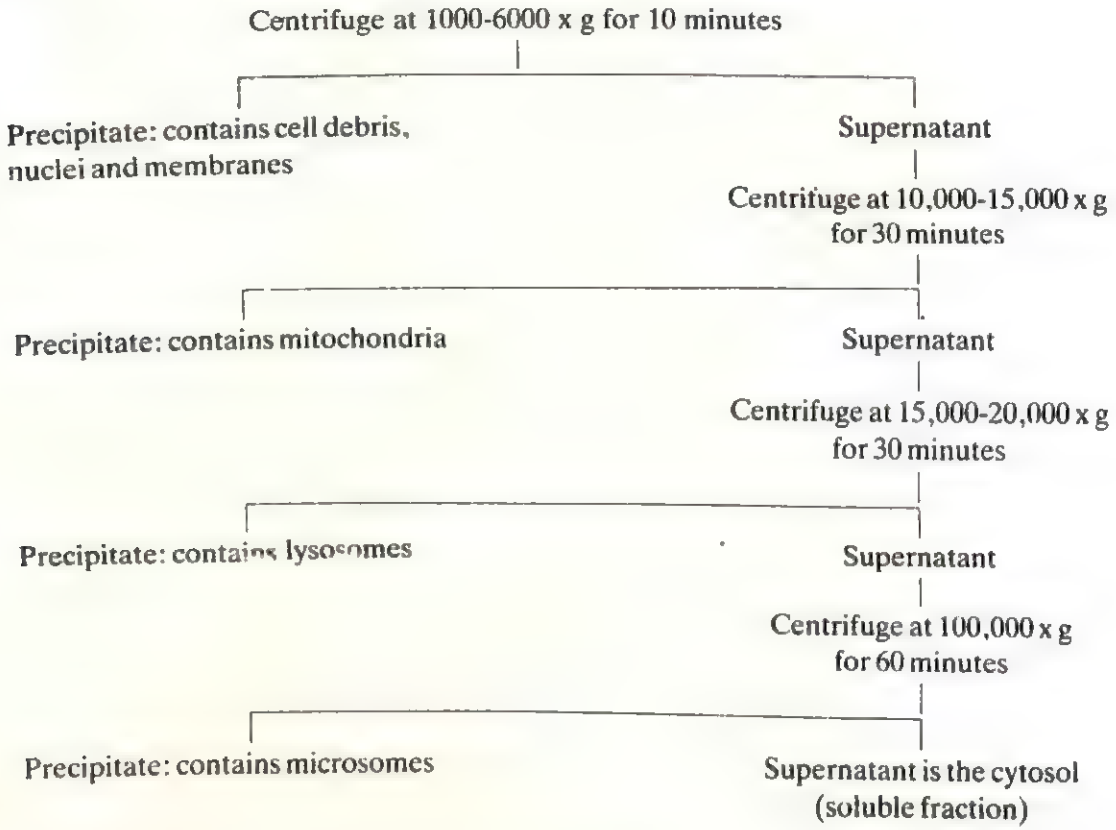
THE living tissues are able to bring about hundreds of chemical reactions every minute of their life to obtain energy, to synthesize the protoplasmic and other structural materials, to absorb from the external environment the substances required for life and to eliminate into the environment the unwanted material from within. Many of these reactions are either not possible at all outside the tissue or can be brought about only by employing reagents that are incompatible with life (e.g.: strong acids or alkalies) and extreme temperatures (boiling point of water and above). If a protein has to be hydrolyzed to amino acids, it has to be boiled with fairly strong acids for several hours. On the side of synthesis, at best, small peptides can be synthesized by purely chemical means. Synthesis of proteins and other complex substances by using only chemicals is not possible at all. But in the tissues, not only are these reactions possible; but they take place under extremely mild and absolutely physiological conditions *viz.*, pH of 7.4 and temperature 37°C. This is possible on account of certain catalysts produced by living cells. Berzelius (1837) referred to the substances responsible for the fermentation of sugars as 'ferments'. The name 'ENZYME' (meaning 'in yeast') was first used by Kuhne (1878). Louis Pasteur (1860) postulated that enzymes of yeast are inextricably linked with the structure and life of yeast cells. Edward Buchner (1897) however succeeded in extracting the enzymes from yeast cells and demonstrated their activity outside the cells. J.B. Sumner (1926) isolated urease enzyme in a crystalline form from jack bean extracts. He suggested that it was protein in nature. During the early 1930's, Northrop crystallized several enzymes — pepsin, trypsin, and chymotrypsin — and confirmed that enzymes are proteins.

Definition: Enzymes can be defined as soluble, colloidal, organic catalysts which are produced by living cells but are capable of acting independently of the cells. All enzymes are protein in nature without any exception and exhibit all properties of the proteins. They are water soluble; precipitated by the usual protein precipitating reagents like alcohol, ammonium sulfate and alkaloidal reagents; they are non-dialyzable, amphoteric, have isoelectric points and have a nitrogen content of about 16%. Extreme alterations of pH and high temperatures denature the enzyme protein and thus make it inactive.

Some enzymes are also capable of combining with non-protein prosthetic groups to form conjugated proteins and several enzymes are active only in the conjugated form. The protein part of the enzyme is then called the 'apoenzyme' and the non-protein prosthetic group is called 'coenzyme'. The conjugated active form is called the 'holoenzyme'.

Intracellular location of enzymes: The microscopic structure of the cell has already been described. The particulate components of the cell can be separated by ultracentrifugation and their enzymes studied individually. After rupture of the cell membranes of the tissue, a

suitable buffer is added and the contents are centrifuged at varying speeds. The centrifugal force is expressed as a multiple of the gravitational force 'g'.



The enzymes in each of these fractions can now be studied.

The enzymes seem to be distributed in a set pattern in the different structures of the cell and the pattern seems to be the most efficient for the functioning of the cell. In the rat liver cells, for example, the enzymes concerned with glycolysis (conversion of glycogen and glucose to pyruvic or lactic acid) are present in the cytoplasm. The enzymes of citric acid cycle and oxidative phosphorylation (oxidation of acetate molecule in the citric acid cycle yielding energy in the form of energy-rich phosphates) are located in the mitochondria. The nucleus and microsomes are also rich in certian enzymes which function mainly in those structures. The lysosomes are seats of several hydrolytic enzymes.

Histochemical methods are also applied to thin sections of the tissue to locate the enzymes in the different sub-cellular fractions. The section is treated with a suitable substrate for the enzyme under study. Products are formed at the site of enzyme action. If the products are colored and insoluble, they remain at the site of formation and can be viewed under the microscope. Alternately, suitable methods of fixing and staining the products differentially from the tissue are available. Acid and alkaline phosphatase, monoamine oxidase and some dehydrogenases can be studied in this manner.

It is possible that much of the protein content of a cell is on account of the thousands of enzymes contained in it.

Extracellular enzymes: While all enzymes are produced in the living cell, some enzymes are secreted out from the cell and function outside the cell of origin. They are called the extracellular enzymes and consist mainly of the digestive enzymes. They are secreted by the cells of the salivary glands, gastric glands, pancreas and intestinal glands, and contain enzymes which are used in the digestion of food materials e.g.: salivary amylase, gastric pepsin and pancreatic lipase.

Purification of enzymes: This is relatively easier in case of extracellular enzymes since the secretion from the gland can be collected and purified. In case of intracellular enzymes, the enzyme has to be first liberated from the cell. This is done by rupturing the cell wall by grinding the tissue with sand or by grinding in, what are known as, homogenizers. The cell wall also ruptures by alternate freezing and thawing of the tissue or by dehydration brought about by grinding with acetone and filtration whereby the acetone with the dissolved cell lipid is removed leaving the protein material with the enzymes.

Subsequently the enzyme present in the crude preparation obtained by one of the above methods is purified by following methods similar to protein purification. Extraction with solvents like glycerol or suitable buffers, dialysis, differential precipitation with various salts, absorption onto substances such as alumina or kaolin, electrophoresis and chromatography are all used employing physiological temperature and pH range to prevent denaturation of the protein. Using such methods, several enzymes had been purified and obtained in a crystalline form.

Free Energy of Activation and the Effects of catalysis:

A chemical reaction $S \rightarrow P$ (where S is the substrate and P the product or products) will take place when a certain number of S molecules at any given instant possess enough energy to attain an activated condition called the 'transition state', in which the probability of making or breaking a chemical bond to form the product is very high. The transition state is at the top of the energy barrier separating the reactants and products. The rate of a given reaction will vary directly as the number of reactant molecules in the transition state. The 'Free Energy of Activation' is the amount of energy required to bring all the molecules in 1 gram-mole of a substrate at a given temperature to the transition state.
A rise in temperature, by increasing thermal motion and energy, causes an increase in the number of molecules in the transition state and thus accelerates a chemical reaction. Addition of an enzyme or any catalyst can also bring about such acceleration. The enzyme combines transiently with the substrate to produce a transient state having a lower energy of activation than that of substrate alone. This results in acceleration of the reaction. Once the products are formed, the enzyme (or catalyst) is free or regenerated to combine with another molecule of the substrate and repeat the process.

Fig. 8-1 shows the changes in the free energy of activation in the presence of an enzyme. Though there is change in the free energy of activation resulting in an accelerated reaction, the over-all free energy change of the reaction remains the same whether the reaction is catalyzed by an enzyme or not.

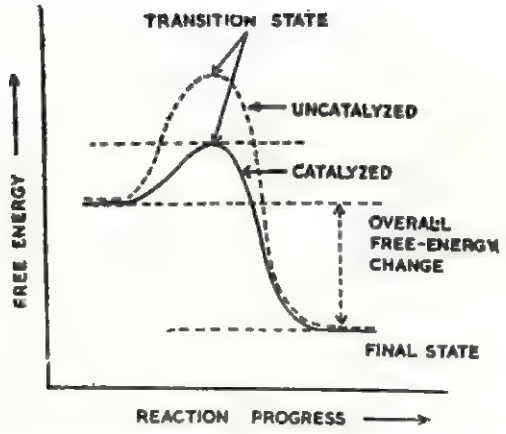


Fig. 8-1. Free energy changes in catalyzed and uncatalyzed reactions

Mechanism of action of enzymes: Wurtz (1880) added the proteolytic enzyme, *papain*, which is water soluble to the insoluble protein, *fibrin*, and then subjected the mixture to repeated washing. Even so, proteolysis of fibrin continued, showing that papain combined with fibrin immediately on addition and could not be removed by washing.

O'Sullivan and Tompson (1890) showed that the enzyme, *invertase* which is normally destroyed by heat, can resist higher temperatures in the presence of its substrate, *sucrose*.

Emil Fischer (1890) suggested that glycosidases which act on glycosides are highly stereospecific with respect to their substrates and that this can be explained only on the basis of similarity in the shape of the enzyme molecule and the substrate molecule which enables them to fit into each other like a *lock and key*.

Peroxidase enzyme has characteristic absorption bands when examined spectroscopically. If H_2O_2, its substrate, is added to it, the bands show an immediate shift, indicating the formation of a new compound.

$$\text{Peroxidase} + H_2O_2 \longrightarrow (\text{Peroxidase-}H_2O_2).$$

If to this, a suitable hydrogen donor is added, the absorption bands of the original enzyme are restored.

$$(\text{Peroxidase–}H_2O_2) + MH_2 \longrightarrow \text{Peroxidase} + M + 2H_2O$$

Finally, it was Michaelis and Menton (1913) who established the theory of formation of an *enzyme-substrate complex* and worked out the kinetics of such complex formation and its subsequent dissociation into products.

According to Michaelis-Menton theory, the enzyme combines with the substance on which it acts (called the 'substrate') to form an enzyme-substrate complex. From this, enzyme is liberated and the substrate is broken down into the products of the reaction.

$$E \text{ (enzyme)} + S \text{(substrate)} \longrightarrow ES \text{ (Enzyme-substrate complex)}$$
$$ES \xrightarrow{\text{Enzyme}} E + \text{Products}$$
$$\therefore S \xrightarrow{\text{Enzyme}} \text{Products.}$$

The ES-complex is also called the "*Michaelis' complex*". By bringing the reactants together at the active site, there is a tremendous increase locally (on the enzyme molecule) in the concentration of the reactants. The concentration may be several thousand times the concentration in the rest of the solvent. This phenomenon of approximation, therefore, enhances the rate of reaction several thousand times.

Destabilization: Binding of the substrate at the active site also results in the loosening or destabilization of certain chemical bonds in the substrate and facilitates ready breakdown at the bonds. This is on account of the catalytic action of certain groups of the enzyme like –COOH, $-NH_2$, –OH and –SH at the active site. The substrate binding brings the substrate into close proximity to some of these groups in the enzyme.

For the combination with the substrate, each enzyme is said to possess one or more active sites where the substrate can be taken up. The active site usually consists of the hydroxy-group of serine, the imidazole group of histidine or the sulfhydryl group of cysteine. The conformation of the enzyme molecule will be such as to expose the group of combination (fit) with the substrate molecule. In other cases the conformation may not be present, but will be induced by a rearrangement of protein molecule on contact with the substrate (induced fit). The concepts involved in the active site and formation of ES complex are presented in Fig. 8-2.

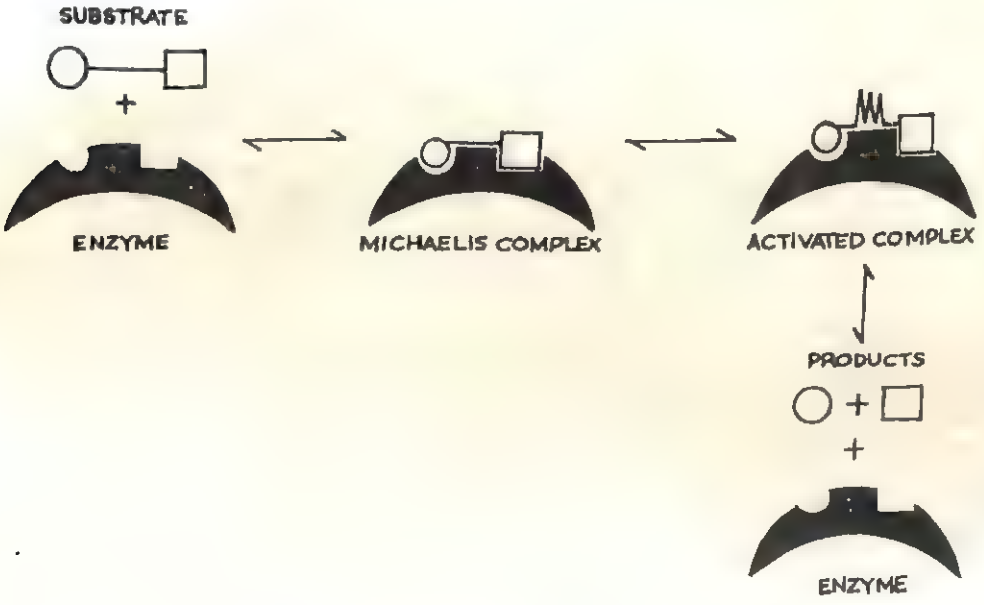


Fig. 8-2 Enzyme Action

It is also possible that the active site (catalytic site) is different from the binding site in which case they are situated closely together in the enzyme molecule.

A few examples of active site in specific enzymes will illustrate the point.

Carboxypeptidase: The active centre contains two tyrosine residues. Acetylation of the tyrosine will abolish the peptidase activity.

Alkaline Phosphatase: The active centre contains a serine moiety which helps in binding as organic phosphate (glucose-6-phosphate). The serine –OH becomes esterified and free glucose is released. In the next step, the phosphate ester with serine is hydrolyzed and inorganic phosphate is set free.

Esterase: This group of enzymes also have –OH of serine and also the nitrogen of histidine at the active site. There is a shift of the hydrogen from the –OH to the histidyl nitrogen to facilitate the E-S complex formation. The overall reaction will be–

$$RCOA + BOH \rightarrow RCOB + AOH$$

The steps are shown in Fig. 8-3.

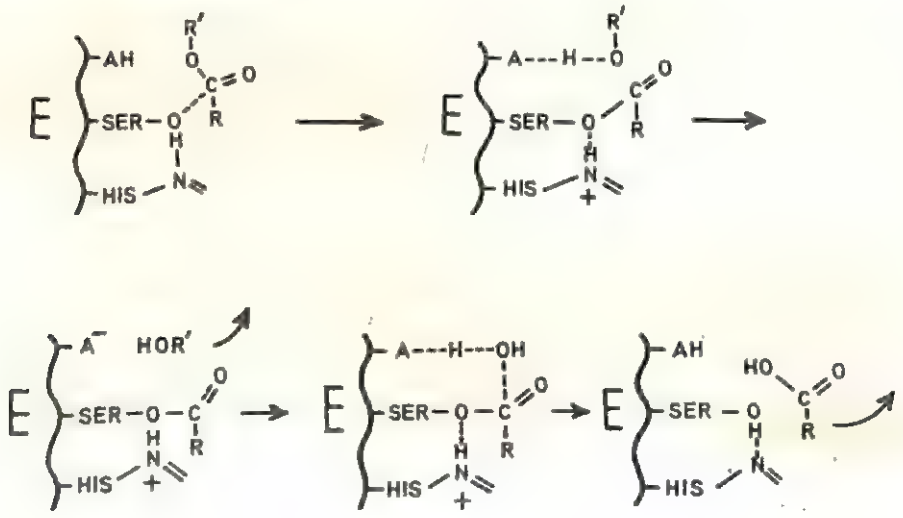


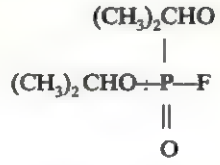
Fig. 8-3. Action of Esterase

Chymotrypsin: Its esterase activity is explained by a similar mechanism. It has a serine and two histidyl residues at the active site. The histidine molecules are far apart in the primary polypeptide structure. They are brought into proximity between themselves and also in proximity to a serine molecule in the folding of the molecule.

Phosphoglucomutase: The active site contains histidine, serine and aspartic acid.

Choline Esterase: Serine is the active centre. This can be blocked by combination with

diisopropylfluorophosphate (DFP) whose structure is as follows:



The DFP firmly combines with the serine. A small peptide containing the serine DFP can be separated out from the enzyme molecule and identified.

Pancreatic Ribonuclease: The structure of the enzyme had been completely worked out. It has 124 amino acids. Histidine is present in positions 12 and 119. The peptide chain is so organized that these two histidyl residues are brought close together by the secondary folding of the moleucle resulting from four disulfide bonds. A cleft is formed at the active site to enable substrate binding. The histidyl nitrogen will form bonds with the –OH of the second carbon of a ribose and with the phosphate -3', 5' -diester linking the 5th carbon of the ribose to the 3rd carbon of the next nucleotide. The phosphate diester is broken. A transient

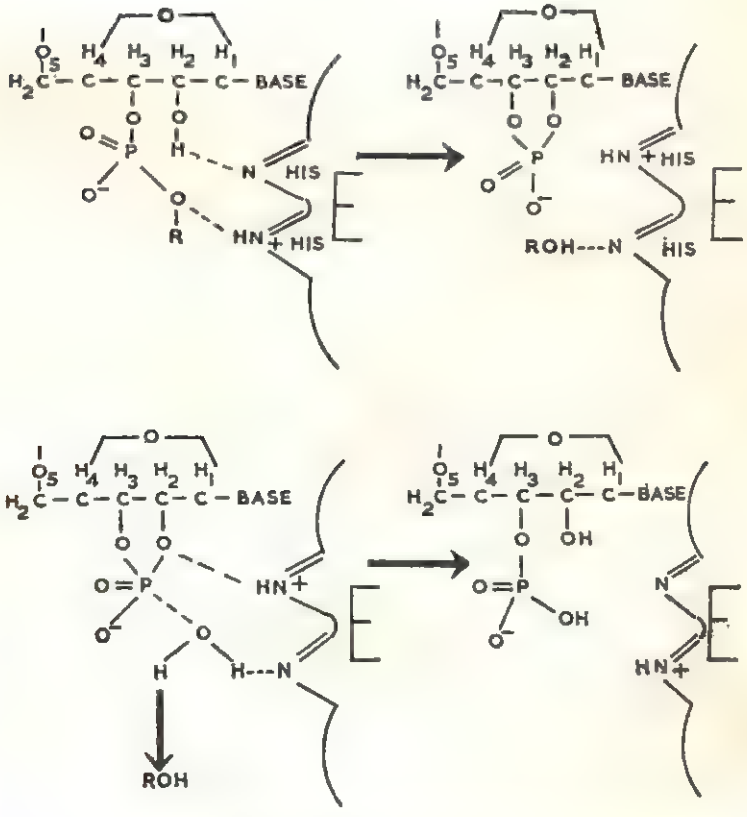
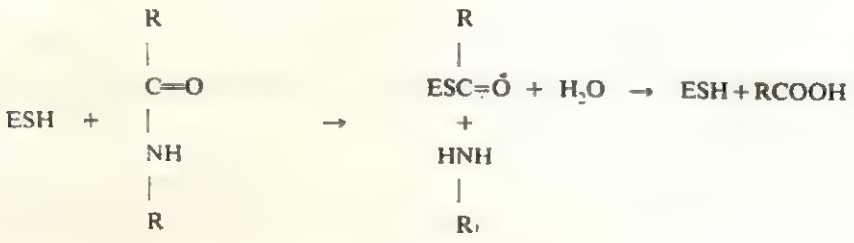


Fig. 8-4. Mode of action of Pancreatic Ribonuclease.

cyclitization of the phosphate with the C-2 of the now released ribose will occur. This is further hydrolyzed and the histidyl residues which participate in these steps by contributing hydrogen get restored to their original state. These changes are schematically represented in Fig. 8-4.

Papain: This is an enzyme in which cysteine is the active centre and takes part in splitting the peptide bond thus:



Lysozyme: The active site contains glutamic and aspartic acids, the free –COOH groups of which bring about a cleavage of the glycosidic linkage in the polysaccharide molecule. A portion of the polysaccharide molecule (consisting of six monosaccharide units) comes into intimate contact with the active site during the process.

Coenzymes: Some enzymes are only active in the presence of certain organic substances which are termed the coenzymes. Unlike the enzymes, the coenzymes are non-protein, dialyzable, organic substances of known chemical composition, in most cases being derivatives of substances recognized as vitamins. The enzymes which are inactive without the coezyme are called the 'apoenzymes' and together with the coenzymes they are called the "holoenzymes'. The coenzymes seem to function by helping in the carriage or transport of one of the products of the enzyme reaction. The coenzyme is liberated with one of the products and the other product is liberated separately into the solution from the enzyme. This association of the coenzyme with one of the products is only transitory. The product may be transferred to some other acceptor which may be even another enzyme system, or may be liberated into the medium, setting free the coenzyme for again participating in the original reaction. This is schematically represented in fig. 8-5.

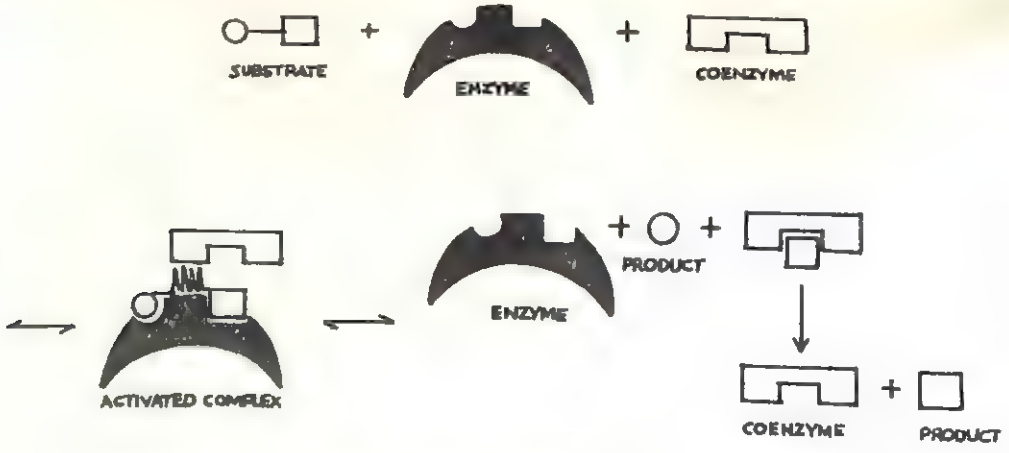


Fig. 8-5. Function of Coenzymes

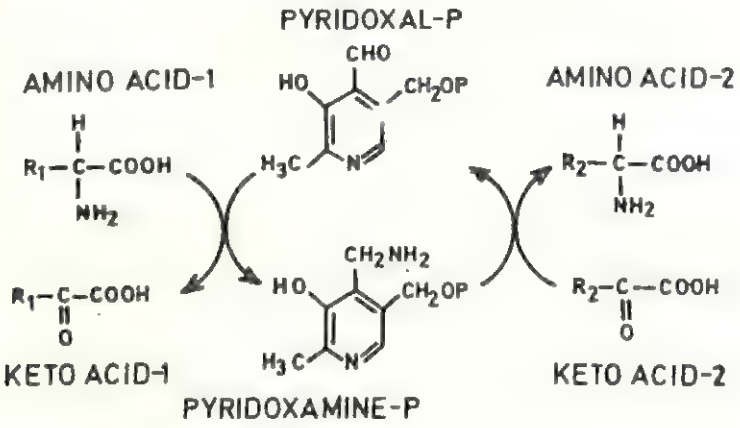
Some of the common coenzymes and their functions are listed in Table 8-1

TABLE 8-1

List of some common coenzymes

Vitamin from which derived	*Name*	*Abbreviation*	*Atom or group transferred*	*Enzymes with which acting*
Niacin	Nicotinamide-adeninedinucleotide (coenzyme–1)	NAD	Hydrogen	Dehydrogenases
	Nicotinamide-adeninedinucleotide Phosphate (Coenzyme-II)	NADP	"	"
Riboflavin	Flavin mononucleotide, Flavin-adenine dinucleotide	FMN	"	"
		FAD	"	"
			"	"
———	Heme	—	Electron $Fe^{+++}+Electron \rightarrow Fe^{++}$	Cytochromes Catalase Peroxidase
Lipoic acid	Lipoic acid	—	Hydrogen and acyl groups	Pyruvic acid dehydrogenase (decarboxylase)
Pantothenic acid	Coenzyme-A	HS–CoA	Acyl group	Thiokinase
Thiamine	Thiamine pyrophosphate	TPP	Aldehyde group Keto group	1) Oxidative decarboxylation of Pyruvic acid. 2) Transketolase
Pyridoxine	Pyridoxal Phosphate	—	Amino group CO_2	1) Transaminases 2) Aminoacid decarboxylases
Biotin	Biotin	—	CO_2 transfer	β-Carboxylase
Folic acid	Tetrahydrofolic acid	THF	Formyl group	—
Cyanocobalamin	5, 6 dimethyl Benzimidazole, Cobamide	—	Carboxyl group	—
———	Adenosinetriphosphate	ATP	Phosphate	Hexokinase Triosekinase
———	Cytidinediphosphate	CDP	Phosphoryl-choline	—
———	Uridinediphosphate	UDP	Sugar, Uronic acid and isomerization of sugars	—
———	S-Adenosylmethionine	—	CH_3	—

The role of pyridoxal phosphate in transamination reactions is a good example.



Activators (ionic): Some enzymes require for their activity the presence of small amounts of specific inorganic ions, *eg:* many of the phosphorylation reactions require Mg^{++} ions; coagulation requires Ca^{++}; Zn^{++} ions are required for carbonic anhydrase action; Fe^{+++} and Cu^{++} ions are required for some oxidative reactions. The ions may help by joining with groups on the substrate molecule (chelation) and bringing them together with enzyme or by actual participation in electron transfer reactions.

Zymogens (proenzymes): Some enzymes are produced by the living cells in an inactive form. They are called the zymogen forms or proenzymes. They are subsequently activated and converted to zymase form. The activation is brought about by specific ions or by other enzymes which are of proteolytic (protein hydrolyzing) nature; *eg:*

Pepsinogen + H^+ (Zymogen) from gastric juice.	⟶	Pepsin (Zymase)
Trypsinogen (Zymogen) from pancreatic juice.	⟶ Enterokinase	Trypsin (Zymase)

The enterokinase is itself a proteolytic enzyme which removes a small peptide from trypsinogen and probably that exposes the hitherto masked active site. The action of H^+ ion on pepsinogen is similar.

Once small amounts of pepsin or trysin are formed, they themselves further catalyze the conversion of the proenzymes into active pepsin or trypsin as the case be. This is known as autocatalysis

Isoenzymes (Isozymes): Some emzymes having similar catalytic function but obtained from different sources exhibit different physical and chemical characteristics eg: different electrophoretic mobilities, amino acid composition and immunological behaviour. They are called isoenzymes or simply isozymes. The *lactic dehydrogenase* from heart muscle and skeletal muscle seem to differ from one another and contian two components H and M in different amounts. Five isozymes of this are isolated to date H_4, H_3M, H_2M_2, HM_3 and M_4. (The number following H or M indicates the number of the H or M present per molecule of the enzyme). The lactic dehydrogenase of skeletal muscle is predominantly M_4 and in cardiac muscle it is H_4. The synthesis of the M and H polypeptide chains is genetically regulated by two different genes. Hence the type of isozyme present in each tissue is genetically regulated.

The two enzymes have different K_m values and different V_{max} values. The skeletal muscle enzyme (M_4) has a low K_m for pyruvate and a high rate of pyruvate ⟶ lactate conversion. The cardiac enzyme (H_4) has a high K_m for pyruvate and a low pyruvate ⟶ lactate conversion rate. As a result, in skeletal muscle, pyruvate is rapidly converted to lactate and sent out into circulation, whereas, in cardiac muscle, pyruvate is oxidized areobically in the citric acid cycle.

Creatinine kinase has two subunits - B and M. BB is the form present in brain (creatinine kinase$_1$ CK_1). BM (CK_2) is present in the myocardium and MM (CK_3) is present in skeletal muscle as well as myocardium.

Measurement of enzyme activity: The enzymes are present only in very minute quantities in any cell or tissue and will be difficult to measure in absolute terms of weight. But their activity can be measured using the substrates on which they act under optimal and standardized conditions. By international convention, one unit of any enzyme is that amount that will catalyze the transformation of one micromole of the substrate per minute under specific conditions.

Specific activity of an enzyme is the number of units of enzyme present in 1 mg of enzyme protein.

Turnover Number: The number of substrate molecules tranformed per minute by a single enzyme molecule is defined as the 'Turnover Number' of that enzyme. Carbonic anhydrase has the highest - 36,000,000.

Enzyme specificity: While catalysts, in general, can function for several chemical reactions, enzyme catalysis is highly speicfic and each enzyme is capable of bringing out only one or a small group of reactions. Thus specificity is an important criterion of enzyme action. The specificity may be of various types:

1. Reaction specificity: Different enzymes bring out different reactions on same substrate:

Eg: $RCH.NH_2COOH$ (Amino acid) $\xrightarrow[\text{oxidase}]{-2H+H_2O}$ $RCOCOOH+NH$

$RCH.NH_2COOH$ (Amino acid) $\xrightarrow[\text{Decarboxylase}]{}$ $RCH_2NH_2+CO_2$

2. *Substrate specificity:* This is of several types.

(a) Absolute specificity:

$$\text{Urea} + H_2O \xrightarrow{\text{urease}} 2NH_3 + CO_2$$

$$CO_2 + H_2O \xrightarrow{\text{Carbonic anhydrase}} H_2CO_3$$

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

(b) Relative specificity: D-Aminoacid oxidases act on all D-amino acids, but they can bring about oxidative deamination of D-tyrosine very rapidly and less so the other D-amino acids.

(c) (i) Group specificity: Several enzymes hydrolyze polypeptides, but each has a preference for peptide linkages involving sepcific sites of polypeptide chain or peptide bonds involving specific amino acids.

Eg: Carboxypeptidase: Acts preferentially on the peptide bond immediately next to free carboxy end of polypeptide chain.

Aminopeptidase: Acts preferentially on the peptide bond immediately next to free amino end.

Pepsin: Acts preferentially on peptide linkages involving tryptophan, tyrosine, phenylalanine and leucine.

Trypsin: Acts best on peptide linkages involving arginine and lysine.

(ii) Phosphorylase acts only on the 1, 4-glycosidic linkages of glycogen while the branching enzyme acts on the 1, 6-glycosidic linkages of glycogen

(d) Stereo specificity on account of isomerism of substrate: The L-amino acid oxidase and D-amino acid oxidase are distinct enzymes which act only on the L and D-amino acids respectively. D-glucose oxidase can similarly act only on D-glucose and is inactive on its L-isomer. In fact all the enzymes of glycolysis act only on the D-isomers of their respective substrates.

Salivary amylase acts on the alfa 1, 4-glycosidic linkages and is inactive on beta glycosidic linkages.

This specificity may be stretched to the products also.

Thus succinic acid dehydrogenase will act on succinic acid to produce always the trans isomer, fumaric acid, and never the cis isomer (maleic acid).

The specificity can be explained on the basis of a three point attachment of the substrate to the enzyme. The substrate 'S' in the figure 8-6 can combine with the enzyme only in one position. If the action of the enzyme, say dehydrogenation, takes place between positions A and B, represented as square and triangle in Fig. 8-6 then the action is confined to group A_1 though A_1 and A_2 are identical. This is exemplified by the action of the enzymes aconitase and isocitrate dehydrogenase on citric acid, using labelled oxaloacetate.

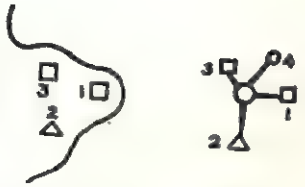
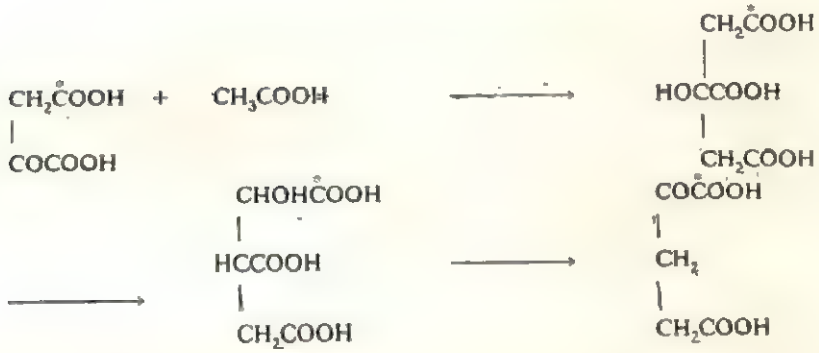


Fig. 8-6. Three point attachment of substrate to enzyme



The labelled $\overset{*}{C}$ derived from the –COOH adjacent to $-CH_2$ group of oxaloacetate is always located in the –COOH attached to the alfa keto group of alfa-ketoglutaric acid. Thus the aconitase and isocitric dehyrogenase act always on the CH_2COOH derived from the oxaloacetate and not on the CH_2COOH derived form acetate.

Physical factors altering enzyme activity:

The action of various factors like temperature, concentration of the enzyme, substrate etc., can all be explained on the basis of kinetic theory or collision theory. For any reaction to occur, two conditions have to be satisfied. i) the reacting molecules will have to collide with each other. All molecules above the absolute temperature (–273°c) are in perpetual motion. The molecular movement increases with rise in temperature. A rise in temperature also increases the kinetic energy of the molecules. Increasing the concentration of the reactant molecules, likewise, will increase the chance for collision between the molecules. ii) the reactant molecules must have sufficient energy to overcome the energy barrier for the reaction. Enzymes are said to lower this energy barrier and initiate the reaction.

1. Contact between the enzyme and substrate: The enzyme being a protein forming a colloidal solution, the substrate also must be a water soluble substance. If the substrate is a lipid it must be emulsified to enable it to come into contact with the enzyme. Thus pancreatic lipase can act on lipids in the gastrointestinal tract only when the lipid is emulisfied by bile salts. In obstructive jaundice where bile is absent in the intestinal tract, fat is excreted in feces undigested.

2. Concentration of the substrate: Keeping all the other things constant, an increase in the substrate concentration increases the enzyme activity till a maximum is reached. Further increase in substrate concentration does not increase rate of reaction. The phenomenon is explained thus—as concentration of substrate is increased, the substrate molecules combine with all available enzyme molecules at their active sites till no more active sites are available. Once this stage is reached, substrate is required only to replenish the sites when the products are liberated and cannot increase the rate of reaction further. (See figure 8-7a).

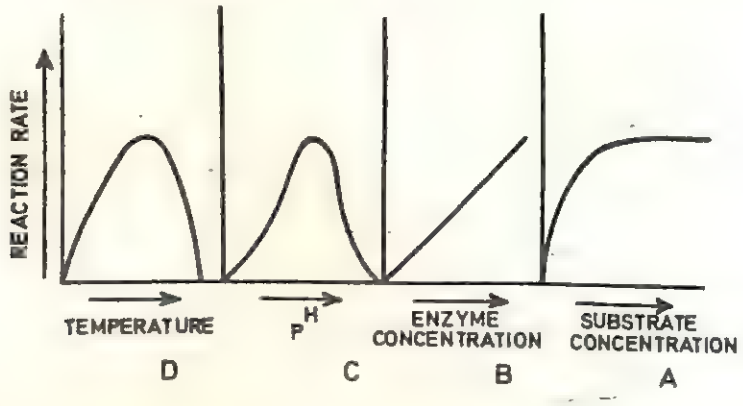


Fig. 8-7. Factors affecting reaction rate

The Michaelis Menton Constant: It is possible to mathematically evolve a relation between the substrate concentration and the velocity of reaction.

$$v = \frac{V(S)}{K_m + (S)}$$

Where v = velocity at a given concentration of substrate.
V = maximal velocity possible with excess of substrate.
(S) = concentration of the substrate at velocity v.
K_m = Michaelis constant for the enzyme.

It is possible to so choose (S) as to make $v = \frac{1}{2} V$. Then $V/2 = \frac{V(S)}{K_m + (S)}$

dividing by V $\therefore \frac{1}{2} = \frac{(S)}{K_m + (S)}$

$$\therefore K_m + (S) = 2(S)$$
$$\therefore K_m = (S)$$

Hence K_m, the Michaelis constant, can be defined as the concentration of the substrate when the velocity of the enzyme reaction is half the maximal possible. The K_m value varies from enzyme to enzyme and is used in characterizing the different enzymes.

For most enzymes, the K_m varies between 10^{-1} and 10^{-6}M. It varies with the environmental conditions of the enzyme such as temperature and ionic strength. The K_m can be considered as the concentration of the substrate at which half the active sites of the enzyme are occupied by the substrate. It is also equal to the dissociation constant of the ES-complex under certain conditions and is hence a measure of the strength of the ES-complex. A high K_m value indicates weak binding between the enzyme and the substrate, whereas a low K_m indicates strong binding.

The maximal velocity (V or Vmax) is a measure of the *turnover number* of the enzyme.

Lineweaver-Burke Plot: Another useful relationship in the study of enzymes is the relation between the reciprocals of substrate concentration and velocity. By inverting Michaelis-Menton equation, we get

$$\frac{I}{v} = \frac{K_m+(S)}{V(S)} \quad \text{or} \quad \frac{I}{v} = \frac{K_m}{V} \times \frac{I}{(S)} + \frac{(S)}{V(S)}$$

$$= \frac{K_m}{V} \times \frac{I}{(S)} + \frac{I}{V}$$

The equation is similar to $Y = aX + b$ which gives a straight line plot. Here $Y = I/v$ and $X = I/(S)$. $a = K_m/V$ and $b = I/V$.

If therefore I/v is plotted as a function of I/(S), it yields a straight line whose intersection of the Y-axis gives I/V and the slope of the line gives K_m/V. (see Fig. 8-8). On extension of the line to the negative side, it cuts the X-axis at a point equal to $-1/K_m$.

The Lineweaver-Burke plot is particularly useful in evaluating inhibitors. A competitive

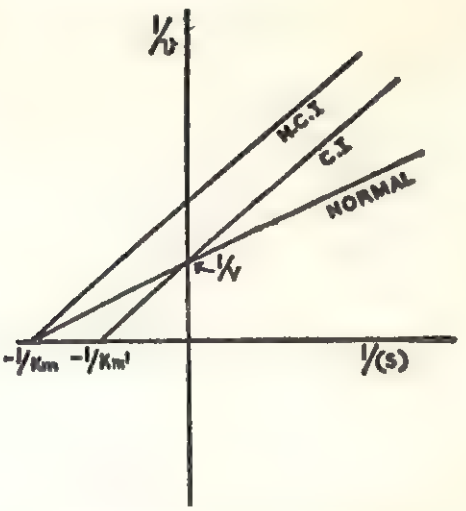


Fig. 8-8 Lineweaver-Burke Plot.

inhibitor will increase the slope of the plot and will intercept the X-axis at a point nearer zero, but it cuts the Y-axis at the same point as without an inhibitor. This means that I/V (and hence the maximum velocity) is not altered but the K_m value is increased since $-I/K_m$ is decreased. On the other hand, a non-competitive inhibitor lowers the maximal velocity (I/V is increased), but does not alter the K_m. These concepts are illustrated in Fig. 8-8.

3. *Concentration of the enzyme:* Within reasonable limits, the rate of an enzyme reaction steadily increases with increasing concentration of the enzyme. The relationship is linear, showing a direct proportionality (Fig. 8-7 b).

4. *Concentration of the products:* Accumulation of products of reaction causes a lowering of the enzyme activity. This is prevented in nature by prompt removal of the products from the site of formation. Eg: absorption of the products of digestion from gastrointestinal tract into the blood stream.

5. *pH:* Some enzymes act best in an alkaline medium; others in an acid medium. For every enzyme there is a pH where it acts at its best and this is its optimal pH. The optimum pH for pepsin is around 2.0 while that of trypsin varies from pH 8.0 to 9.0. It is probable that pepsin attacks the substrate molecules best if they carry a positive charge while trypsin attacks the negatively charged substrate molecules. For most biological enzymes the optimum pH is around 7.4 (Fig. 8-7 c).

6. *Effect of temperature:* All chemical reactions get accelerated with rise in temperature, whether mediated by catalyst or not. In case of enzyme activity also this holds good up to a certain increase in temperature (say 50°C). Above that temperature, another process– namely the denaturation of the enzyme protein sets in and rapidly decreases the enzyme activity annulling the general beneficial effect of heat on chemical reaction. The enzyme reactions altogether cease at a temperature of 70 to 80°C. If the temperature is further raised, the enzyme becomes totally denatured and remains inactive even if temperature is brought down subsequently. This is an important means of distinguishing an enzyme catalyzed reaction from other catalytic reactions. All catalytic activity is lost on boiling if catalysis is enzymic.

An enzyme becomes less active when cooled and is altogether inactive at 0°C. But the activity is regained if the temperature is raised again. Thus it is a reversible inactivation. Enzymes can be stored for years by keeping in a frozen state. They regain full activity if they are brought back to laboratory temperature.

The temperature at which an enzyme shows maximum activity is known as the optimum temperature for the enzyme. For most animal enzymes it is around the body temperature 37°C (see Fig. 8-7 d). Some plant enzymes like urease have higher optimum temperature even up to 60°C.

Temperature coefficient–Q_{10}: The ratio of the reaction rate at temperature t +10°C to that at t° is known as the temperature coefficient and is usually 2. That means the rate of reaction is double the initial rate when the temperature is increased by 10°C.

7. *Effect of oxidizing substances:* The activity of many enzymes, particularly the hydrogenases depends on –SH groups. The activity is lost if the –SH groups are oxidized to -S-S- groups. Reducing substances like glutathione and cysteine can reactivate such enzymes.

8. *Radiation:* Exposure to ultraviolet rays, X-rays, beta and gamma rays causes the formation of peroxides which oxidize the enzymes and make them inactive. This is an immediate effect. In addition, they exert effects on the DNA molecules (genes) which will lead to impaired synthesis of the enzymes as a delayed effect.

Enzyme Inhibition:

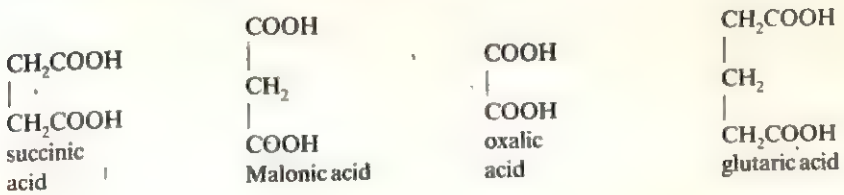
1. *Non-specific inhibition:* Many enzymes like papain, urease and succinic dehydrogenase require for their activity the presence of free -SH groups in their molecules. The addition of glutathione, cysteine, H_2S and traces of HCN activate such enzymes by preventing oxidation of the -SH group. On the other hand, compounds like iodoacetate and P-chloromercuribenzoate (PCMB) combine with -SH groups and thus inactivate the enzymes. Heavy metals like Ag^+, Hg^{++} also combine irreversibly with the -SH groups and inactivate enzymes. Some enzymes which require metallic ions for their activity are inhibited by chelating agents like ethylenediamine tetraacetate (EDTA).

This type of inhibition is irreversible and will make the active site of the enzyme ineffective. Addition of any amount of the substrate does not restore activity.

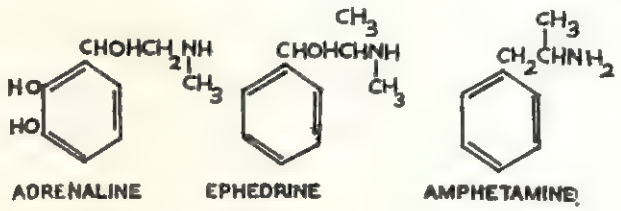
2. *Competitive inhibition:* The pharmacological action of several drugs is dependent on the ability of the drugs to inhibit one or other of the enzymes responsible for the growth and multiplication of microorganisms. The inhibitor, which has close structural resemblance to the substrate, competes with the substrate for the active or binding sites and thus diverts much of the enzyme to form the enzyme-inhibitor complex instead of enzyme-substrate complex. The enzyme-inhibitor complex does not yield any products and hence remains stable, thus preventing further enzyme activity. This type of an inhibition can be reversed by adding excess of substrate which will successfully dislodge the inhibitor molecules from the enzyme. *Uncompetitive inhibition:* When the inhibitor combines with the ES complex instead of E and causes inhibition, it is sometimes called uncompetitive inhibition. The ESI complex cannot form any products. The inhibition is not reversed by increasing substrate concentration.

Some examples of competitive inhibition are considered below:

i) Succinic dehydrogenase can be inhibited by malonate, oxalate and glutarate, all of which resemble the substrate, succinic acid, in structure.

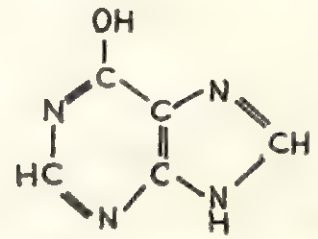


ii) Monoamine oxidase (MAO) oxidizes pressor amines like adrenaline and noradrenaline. Ephedrine and amphetamine, which have similar structure as adrenaline and noradrenaline, inhibit the enzyme and thus prolong the action of the pressor amines.

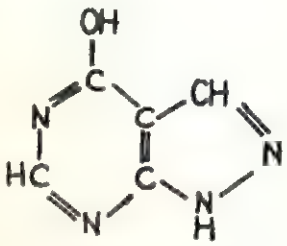


Cheese contains large amounts of tyramine. The oxidation of tyramine also requires MAO. By decreasing the availability of MAO for adrenaline oxidation, cheese also prolongs adrenaline action.

(iii) In gout uric acid accumulates in tissues, and causes symptoms. Uric acid is formed by oxidation of hypoxanthine by xanthine oxidase. Allopurinal has a structural resemblance to hypoxanthine and by competitive inhibition decreases formation of uric acid.



HYPOXANTHINE



ALLOPURINOL

(iv) Higher animals as well as microorganisms require certain natural substances like vitamins, amino acids, purines, pyrimidines etc., which are used as such or after incorporation into other macromolecules. The utilization of these metabolites is dependent on enzymes. The enzymes may be inhibited by substances which have a structural resemblance to the metabolites. The inhibitors are called *"antimetabolites"*, and the type of inhibition is competitive.

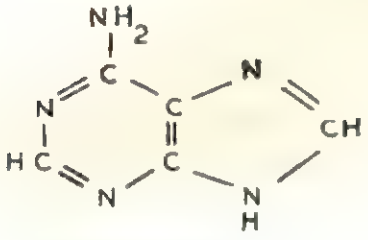
Some of these antimetabolites are produced by living organisms like bacteria, fungi etc., and are then called *"antibiotics"* eg. penicillin, streptomycin, chloramphenicol and tetracycline.

Regulation of Enzyme Activity

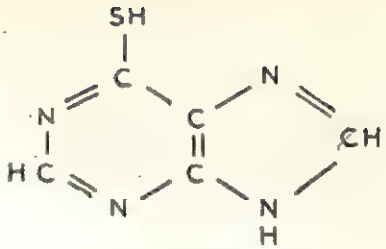
Allosteric enzymes: Some enzyme reactions are regulated by *'effectors'* which can bind reversibly to the enzyme molecule at specific sites other than the substrate-binding site, or the *'allosteric sites'* as they are called. If the binding of the effector causes inhibition of the

Para aminobenzoic acid

Sulfanilamide



ADENINE



MERCAPTOPURINE

enzyme reaction, it is called a *negative effector,* and the process is called *'allosteric inhibition'*. If, on the other hand, the enzyme reaction is activated, it is called a *positive effector* and the process is called *'allosteric activation'*.

The inhibition can be brought about by decreasing the affinity of the enzyme to the substrate (increasing the Km value) or by a decrease in the Vmax. Allosteric activation has the opposite effect - decrease in Km or increase in Vmax. If the effector substance is the substrate itself, it is called *'homotropic effect'*. If it is some other substance, it is called *'heterotropic effect'*.

All allosteric enzymes have two or more subunits and contain more than one binding site for the substrate. The interaction with an effector substance brings about conformational changes in the substrate - binding site. This may result in an inhibition or activation of the catalytic activity.

Inhibition of aspartate transcarbamylase by cytidinetriphosphate (CTP) is an example of allosteric inhibition. CTP has no structural resemblance to aspartate. In fact it is an intermediate in the synthesis of nucleic acids from carbamyl aspartate.

Carbamyl-P ⟶ carbamyl aspartate ⟶ CTP ⟶ Nucleic acid

When CTP accumulates, it inhibits the first reaction and slows down its own synthesis. This is an example of feed-back inhibition of an enzyme by a remote product in a sequence of chain reactions.

This is a process which operates in the normal functioning of the enzymes of many tissues. The end product of a chain or cycle of reactions will usually inhibit the enzyme concerned with the early stages of the chain of reactions with the result that the synthesis will stop till

the level of the end product is again brought down. Thus in the synthesis of cholesterol by liver. increased levels of gastrointestinal absorption of cholesterol cause inhibition of HMG–CoA reductase, an enzyme concerned in the initial stages of cholesterol synthesis in the liver and thus adjusts the total cholesterol made available to the organism from food and by in vivo synthesis.

The step in the multienzyme reactions which is catalyzed by the allosteric enzyme is usually irreversible i.e. once this enzyme reaction occurs, the rest of the reactions in the sequence necessarily follow. Such a reaction is called the "*committing reaction*".

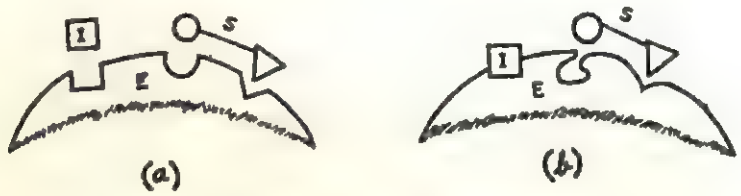
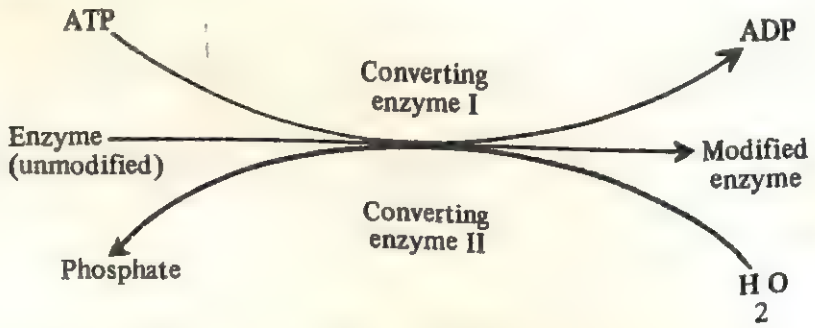


Fig. 8-9. Allosteric inhibition

2. Cascade Systems: Enzyme activity is also regulated by cyclic interconversion of enzyme into two forms - a modified and an unmodified form. The interconversion is brought about by a '*converting enzyme*' which together with the two forms of the enzyme (modified and unmodified) forms a cascade system.



In many of these enzymes, the modification involves phosphorylation of the enzyme at an -OH group of serine, threonine or tyrosine. Examples of such cascade systems include liver phosphorylase, glycogen synthase etc. The convertor enzymes or usually protein kinases and themselves exist in an inactive form and require to be activated by substances like cyclic AMP. The production of cyclic AMP is in turn regulated by an enzyme, *adenylate cyclase*, whose activity is under hormonal influence. The enzyme modified by phosphorylation may become more active (eg. liver phosphorylase) or less active (eg. glycogen synthase). The phosphate added to the enzyme by effector enzyme 1 is removed by effector enzyme 11, which is usually a phosphatase.

Antienzymes: Ascaris Lumbricoides (round worm) which inhabits the human and animal gastrointestinal tract as a parasite survives the action of the digestive enzymes of the alimentary canal by elaborating what are called antienzymes against trypsin and pepsin. Antienzymes also develop in the animal if an enzyme is injected parenterally. The enzyme protein acts as an antigen and stimulates antibody production.

Genetic basis for enzyme synthesis: Since all enzymes are proteins, their synthesis follows the general pattern of protein synthesis and is regulated by genes. For every enzyme, there is said to be one gene (one gene, one enzyme hypothesis); where an enzyme is a complex protein containing more than one protein subunit more than one gene may be concerned in its synthesis. Genetic mutation results in abnormal DNA code and synthesis of an abnormal enzyme protein. Since the abnormal enzyme cannot serve the normal function, a metabolic abnormality occurs and this is transmitted to the progeny. Such transmittable abnormalities of metabolism due to abnormal enzyme molecules are known as 'Molecular diseases' or 'Inborn Errors of Metabolism'. Phenylketonuria, alkaptonuria, pentosuria, glycogen storage disease, galactosemia and cystinuria are but a few of several known molecular diseases.

Regulation of enzyme synthesis: Synthesis of an enzyme can occur as needed by the organism. The presence of metabolite, usually a small molecule, will act as a signal for the particular enzyme or enzymes required for its metabolism to be synthesized. The small molecule may induce the synthesis by itself (induction) or may remove a repressing influence (derepression) brought about by a repressor. Thus Escherichia coli grown on a glucose medium do not have the enzyme beta-galáctosidase. If they are now switched over to a medium containing lactose, they begin to synthesize that enzyme. This is an example of induction, lactose being the inducer for the enzyme. Tryptophan pyrrolase, threonine dehydrase, penicillinase and invertase are a few examples of inducible enzymes.

Microorganisms grown on culture media containing an abundance of one amino acid (say leucine) show a diminished synthesis of enzymes required for the synthesis of that amino acid. The enzymes for leucine synthesis are repressed, and leucine is termed the corepressor. The actual repressor is a macromolecule, a protein, which acts only in the presence of the co-repressor. Removal of leucine from the medium will cause a 'derepression' and allow the enzyme synthesis to occur.

Diagnostic Applications: Plasma contains several enzymes, some of which are functional in the plasma, while others are merely present in plasma due to leakage from tissues. Lipoprotein lipase, psuedocholinesterase and enzymes concerned in the coagulation of blood and the dissolution of the blood clot are enzymes that serve a function in the plasma. Though many other enzymes have no function in the plasma, they are still useful as diagnostic tools. Measurement of their levels in plasma offers valuable information about diseases involving the tissue of their origin. A few examples are given below:-

1. *Lipase:* Plasam lipase levels are elevated in acute pancreatitis and carcinoma of the pancreas. They are decreased in liver disease, vitamin A deficiency and in diabetes mellitus.

2. *Amylase:* Plasma amylase is increased in acute pancreatitis, and in inflammatory conditions of the salivary glands. It is decreased in liver disease.

3. *Trypsin:* This is also increased in pancreatic disease.

4. *Cholinesterase:* Low plasma levels are seen in liver disease, malnutrition and anemia It is increased in nephrosis. The enzyme is inhibited by certain drugs, poisons and insecticides and its estimation in plasma will help in the follow up of cases of poisoning by these substances.

5. *Alkaline phosphatase:* It is increased in rickets, hyperparathyroidism, several diseases and disorders involving bone and in obstructive jaundice. Isoenzymes from bone, liver, placenta and intestine can be separately identified and their individual estimations can pinpoint the viscera that is involved.

6. *Acid phosphatase:* Its origin is from the prostate gland. An elevation of its level in plasma is highly suggestive of prostatic carcinoma.

7. *Transaminases:* Serum levels of glutamic-pyruvic-transaminase (SGPT) and glutamic-oxaloacetic-transaminase (SGOT) are useful in the diagnosis of liver parenchymal damage and myocardial damage respectively. In liver damage, both enzymes are increased, but SGPT increases more. In myocardial infarction SGOT is increased with little or no increase in SGPT.

8. *Lactate dehydrogenase (LDH):* The enzyme is increased in plasma in myocardial infarction, acute leukemias, generalized carcinomatosis and in acute hepatitis. Estimation of its isoenzymes is more useful in clinching diagnosis between hepatic disease and myocardial infarction.

9. *Isocitrate dehydrogenase (ICD):* ICD levels are increased in plasma in liver disease. They are also increased in C.S.F. in menigitis and cerebral tumours.

10. *Creatine phosphokinase (CPK or CK):* The enzyme is mainly present in skeletal muscle, cardiac muscle and brain. It has three isoenzymes formed by combinations of two monomers M (for muscle) and B (for brain).

CK_1 is BB, CK_2 is MB and CK_3 is MM.

In normal individuals MB isozyme forms less than 2% of total plasma CK. In myocardial infarction, this is increased 20 fold and forms 5-20% of total CK.

11. *Ceruloplasmin:* Plasma levels of this enzyme are increased in hepatic disease, infections and pregnancy and are not of much diagnostic significance. But a decrease is of diagnostic value in Wilson's Disease (hepatolenticular degeneration).

Classification and nomenclature: All enzymes are broadly divided into six groups based on their function:

1. Oxidoreductases
2. Transferases
3. Hydrolases
4. Lyases
5. Isomerases
6. Ligases (Synthetases)

1. *Oxidoreductases:* Bring about oxidation-reduction reactions.
   Eg: Alcohol dehydrogenase: glyceraldehyde-3-phosphate dehydrogenase: amino acid oxidase.

$$CH_3CH_2OH + NAD^+ \xrightarrow{\text{Alcohol dehydrogenase}} CH_3CHO + NADH + H^+$$

2. *Transferases:* Facilitate transfer of a group other than hydrogen from one substrate to another. Eg: Choline acetyl transferase, hexokinase, transaminase.

$$CH_3CO\text{–}SCoA + \text{Choline} \xrightarrow{\text{Cholineacetyl transferase}} CH_3CO\text{–Choline} + HS\,CoA$$
(Acetyl-choline)

3. *Hydrolases:* Catalyze hydrolysis.
   Eg: cholinesterase, pepsin and amylase.

$$CH_3CO\text{–Choline} + H_2O \xrightarrow{\text{Cholinesterase}} CH_3COOH + \text{Choline}.$$

4. *Lyases:* Catalyze removal of groups by other mechanisms and usually leave double bonds.

   Eg: aldolase, fumarase and pyruvate decarboxylase.

$$\text{Fructose-1,6-phosphate} \xrightarrow[\text{Aldolase}]{} \text{Glyceraldehyde-3-P} + \text{Dihydroxyacetone-P}$$

5. *Isomerases:* Catalyze conversion to other isomeric forms.
   Eg: Triosephosphate isomerase and glucosephosphate isomerase.

$$\text{D-Glyceraldehyde-3-phosphate} \xrightarrow{\text{Triose isomerase}} \text{Dihydroxyacetone phosphate.}$$

6. *Ligases:* Catalyze linking together of two compouds.
   Eg: Succinic thiokinase, glutamine synthetase and acetyl-CoA carboxylase.

$$\text{L-Glutamic acid} + NH_3 + ATP \xrightarrow{\text{Glutamine synthetase}} \text{L-Glutamine} + ADP + \text{Phosphate}$$

Enzymes are customarily named by adding the suffix 'ase' to the name of the substrate on which it acts–

Name of substrate	*Enzyme*
Lipid	Lipase
Amylum	Amylase

Some enzymes were given names orginally which were not directly related to substrate or action. Eg: Pepsin, trypsin.

A commission on enzymes has recommended to follow a numerical system of classification whereby each enzyme can be identified by 4 numbers - eg: 2,4,6,9 etc., the first number indicating the number of the above 6 classes to which it belongs; the next number representing the sub-class; a third figure indicating further sub-class (sub-sub-class); and the fourth number indicating the serial number of enzyme in that sub-sub-class. The method is cumbersome for routine use, but is very useful in pinpointing a single enzyme without ambiguity. See Table 8-2.

TABLE 8-2

Numerical Classification of Enzymes

	Major Class	*Sub Class*	*Code No.*	*Technical Name*	*Common Name*
1.	Oxidoreductase	Acting on I.-CHOH	I.I.I.I.	Alcohol: NAD oxido-reductase	Alcohol dehydrogenase
	"	4. -$CHNH_2$	1.4.1.3.	L-glutamate: NAD (P) oxido-reductase (deaminations)	L-glutamic dehydrogenase
2.	Transferase	4. glycosyl	2.4.1.1.	α I, 4-glucan: orthophosphate glycosyl transferase	Phosphorylase
	"	7. Phosphate	2.7.1.1.	ATP: D-hexose-phospho-transferase	Hexokinase
3.	Hydrolase	1. Ester	3.1.1.8.	acetylcholine acylhydrolase	pseudocholinesterase
4.	Lyase	1. aldehyde	4.1.2.7.	ketose-I-phosphate aldehyde lyase	aldolase
5.	Isomerase	2. cis-trans	5.2.1.3.	all-trans-retinene II-cis-transisomerase	Retinene isomerase
	"	3. aldolase-ketose	5.3.1.1.	D-glyceraldehyde-3-phosphate ketol isomerase	Triosephosphate isomerase
6.	Ligase	3. C-N	6.3.1.2.	L-glutamate ammonia ligase (ADP)	Glutamine synthetase
	"	4. C-C	6.4.1.2.	Acetyl-CoA:CO_2 ligase (ADP)	Acetyl-CoA carboxylase

ACTIVE TRANSPORT ACROSS MEMBRANES

The biological membranes are impermeable to most polar molecules and help in retaining the ionized metabolites within the cell and prevent them diffusing out. But the cells also require to take in polar nutrients like glucose and amino acids from the environment and to secrete into the environment ions like H^+ and other polar molecules. In several instances, the intake and output are against a concentration gradient and are mediated by specific active transport systems. The entry of some substrates like ATP into organelles in the cell (like mitochondria) also requires active transport across the internal membranes. In most cases an intermediate carrier molecule mediates or facilitates such transport. It is called 'mediated' or 'facilitated' transport. It very much resembles enzyme reactions.

The carrier molecule is comparable to an enzyme and the transported substance to the substrate. The rate of transport increases with the concentration of the substance till a certain level only after which the capacity of the carrier molecule becomes saturated and no further increase in transport occurs. The carrier molecule has a specific site to which the substance combines to form a *carrier-substance complex*. Like enzymes, the carriers also exhibit specificity for the substance transported. D-Glucose and structurally related sugars are readily transported across the erythrocyte cell membrane. D-Fructose and disaccharides like lactose are not transported into the erythrocyte when they are present in physiological concentrations in plasma. Most animal cell membranes show stereospecificity in transporting L-amino acids much faster and in preference to the D-amino acids.

Phenomena like competitive inhibition are also observed in mediated transport across membranes, due to binding to the carrier molecules by substances similar in structure at the binding sites. There is also non-specific or non-competitive inhibition of transport by substances like N-ethylmaleimide which blocks sulfhydryl groups of 2, 4-dinitro-flourobenzene which blocks free amino groups.

All these properties of membrane transport are strongly suggestive that protein molecules in the membrane play a crucial role in the process. The specific proteins serving a role in transport are called *'transport systems' 'Carriers' or 'translocases'*.

Mediated or facilitated transport can be *'active' or 'passive'*. In active transport (1) the substance is usually transported against a concentration gradient i.e. from a lower concentration on one side of the membrane to a higher concentration on the other side; (2) the transport process requires expenditure of energy - usually the breakdown of ATP, and (3) the transport is unidirectional: e.g. in the erythrocyte, sodium ion is transported out of the cell and potassium ion into the cell.

Mechanism of transport: Specific transport proteins say (T) contain specific binding sites for specific substances, say (S). The protein is so positioned on one surface of the membrane that it can readily take up (S) from the medium to form a TS complex. Now the transport protein diffuses across the membrane to the other surface or undergoes a rotation or conformational change so that the binding site faces the other side of the membrane. The substance (S) gets discharged into the medium on the other side of the membrane (see Fig. 8-10). This is passive transport and can occur in either direction depending on the concentration of the substance on either side of the membrane.

In active transport, the breakdown of the TS complex or its formation will require energy and is coupled with an energy yielding system like the hydrolysis of ATP. Such transport can occur against a concentration gradient (see Fig. 8-11).

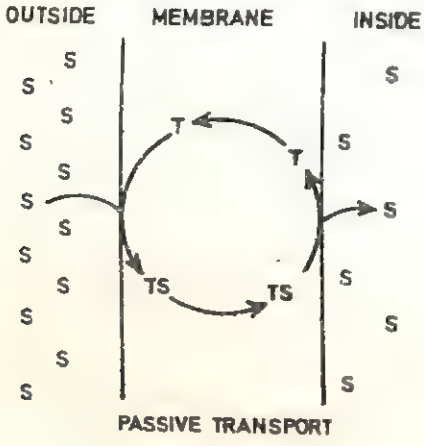


Fig. 8-10

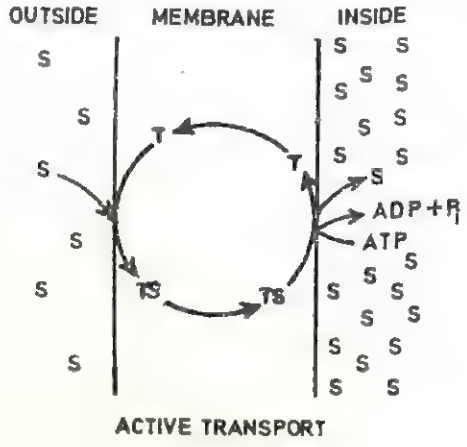


Fig. 8-11

Group Dislocation: Some substances undergo a reversible chemical transformation before they can be transported across a membrane-e.g. glucose is converted to glucose-6-phosphate before it can be transported across some bacterial cell membranes. This is called group translocation. Transport can occur against a gradient. The protein molecule involved in such transport is usually a membrane-bound enzyme.

Transport may be (1) homocellular transport occurring across the cell membrane into or out of the cell or (2) transcellular transport occurring through the cell i.e. the substance is taken up at one side from the medium (say plasma) and extruded from the other side (say into the lumen of the gastric gland). Secretion of H^+ ion by the peptic cell and absorption of substances by the intestinal epithelial cells are some examples. (3) A third variety of transport is 'intracellular transport' –trasport into or out of an intracellular organelle like the mitochondria.

A few specific examples of membrane transport may now be considered.

1. Passive transport systems:

i) *Glucose transport in human erythrocytes:* This is an example of passive mediated transport. D-Glucose, D-mannose, D-galactose, D-xylose, D-arabinose and D-ribose are all transported into the human erythrocyte. The carrier system gets saturated when the outside concentration goes beyond a point and the transport reaches a steady state. Phloretin and trihydroxyacetophenone completely inhibit glucose transport across the erythrocyte membrane.

ii) *ATP-ADP transport in mitochondria:* From cytosol, ADP passes into the mitochondrial matrix where it is phosphorylated to form ATP which passes out into the cytosol. The carrier molecules are specific for ADP-ATP system and do not transport other nucleotide phosphates, nor even AMP. This is also an example of passive transport.

2. Active Transport Systems:

i) *Na^+ and K^+ -transporting ATPase system:* Most of the animal cells maintain a high intracellular potassium concentration and a low sodium concentration against a gradient of high sodium and low potassium of the extracellular fluids. A high intracellular K^+ is needed for the biosynthesis of protein by the ribosomes, activation of certain enzymes like the pyruvate kinase and for the maintenance of the membrane potentials of excitable tissues. The transport is mediated by an enzyme called Na^+, K^+ -ATPase. It has a molecular weight of 250,000 to 300,000. It has one large and one small subunit, the large one extending through the entire thickness of the membrane. The outer surface contains the K^+ binding site. The inner surface contains the Na^+ and ATP binding sites. In the erythrocyte, for every molecule of ATP hydrolyzed, three sodium ions are extruded and two potassium ions are taken in. It is thus essentially a sodium pump. Mg^{++} ions are required for activity of the enzyme. The enzyme seems to act only on the magnesium complex of ATP.

ii) *Glucose absorption by intestinal and renal epithelial cells:* The transport of glucose into the cell seems to be intimately linked with the simultaneous transport of Na^+ into the cell. There appear to be a common carrier molecule for sodium and glucose with two distinct binding sites. The same Na^+, K^+ -ATPase acts in a direction which is the reverse of the sodium pump.

iii) *Amino acid transport by intestinal and renal epithelium:* Different types of transport systems seem to function for different groups of amino acids. High concentration of Na^+ in the external medium favours the transport of glucose as well as amino acids. Glutathione seems to actively participate in the transport.

Amino acid + γ –Glutamylcysteinylglycine
(from environment) (glutathione, from cytosol)

$\longrightarrow$ γ –Glutamylamino acid + cysteinylglycine

γ –Gutamylaminoacid passes into the cytosol and is cleaved to glutamic acid and amino acid. The glutamic acid is reincorporated into glutathione and the cycle is repeated. Energy derived from the breakdown of ATP is required at various stages in the process.

This is also an example of group translocation mechanism.

iv) *Intracellular transport of Ca^{++}*: Transport of calcium ions from the sarcoplasm into the sarcoplasmic reticulum is necessary for the relaxation of muscle. It is brought about by the action of a Ca^{++} transporting ATPase system of the membrane of the sarcoplasmic reticulum and is coupled with the breakdown of one ATP for every two calcium ions transported. Ca^{++} is also taken up by the mitochondria from the cytosol by an energy dependent process which maintains a high intramitochondrial calcium concentration against a low cytosol concentration.

3. Intercellular Contact and Communication

The passage of ions and small molecules between neighbouring cells is facilitated by what are known as '*gap junctions*' which consist of narrow, hydrophilic pores connecting the cytoplasm of adjacent cells. The pores are made up of subunits called '*connexons*' consisting of 6 protein subunits which span the membrane. The subunits can orient themselves in such

a way that they can form a central canal, about 2 nm in diameter, in response to specific chemical stimuli. At other times, they are so oriented that no opening exists between them.

Endocytosis

This is another form of transport whereby extracellular material is incorporated into the cytoplasm. Pinocytosis is one of the methods of endocytosis. Pinocytosis can be a selective process mediated by receptors on the cell membrane or it can be non selective.

Small invaginations are formed on the cell membrane, the edges of which finally fuse to form a vesicle enclosing the extracellular material which now becomes intracellular (see Fig. 8-12). The vesicle migrates into the cytoplasm and gets adherent to a lysosome. The lysosomal enzymes act on the membranous wall of the vesicle to release the contents of the vesicle into the cytoplasm. Receptors, if any, or recycled back to cell surface.

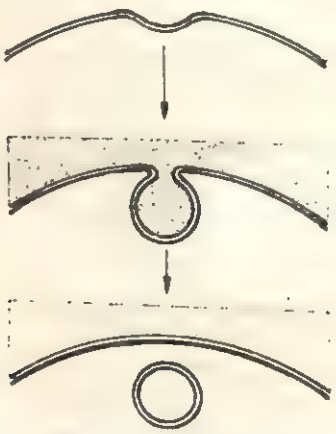


Fig. 8-12. Endocytosis (Pinocytosis)

References:

Atkinson, D.E.: Regulation of Enzyme Action, Ann. Rev. Biochem, 35:85-124, 1966.
Boyer, P.D., Lardy, H. and Myrback, K.: (eds): The Enzymes., Vols. 1-7, 3rd. ed. Acad. Pr. N.Y., 1970-73.
Colowick, S.P. and Kaplan, N.O.: Methods in Enzymology (series of volumes) Acad. Pr., N.Y., from 1955.
Dixon, M. and Webb, E.C.: Enzymes, 2nd, ed. Acad. Pr. N.Y., 1964.
Florkin, M. and Stotz, E.H. (eds): Comprehensive Biochemistry, vols., 12-16, Sec. III, Elsevier, Amsterdam, 1964-66.
Goodwin, T.W., Harris, J.I. and Hartley, B.S. (eds): Structure and Activity of Enzymes: Acad. Pr., N.Y. 1964.
Ingraham, L.L.: Biochemical Mechanisms., Wiley, N.Y., 1962.
Innerfield, I.: Enzymes in Clinical Medicine, McGraw-Hill, Blackiston Div., N.Y., 1960.
Koshland, D.E. Jr. and Neet, K.E.: The Catalytic and Regulatory Properties of Enzymes., Ann. Rev. Biochem 37:359, 1968.
Webb, J.L.: Enzymes and Metabolic Inhibitors, Vols. 1 & 2 Acad Pr., N.Y., 1963, 1966.

9

VITAMINS

INTRODUCTION

THE history of the discovery of vitamins is as fascinating as the vitamins themselves. Takaki, in 1880, was able to prevent the occurrence of beri-beri in Japanese navy by adding meat, vegetables, and evaporated milk to their rations. Scurvy was prevented by the English sailors by addition of limes or lemons to their rations. But neither the causative factor nor the substance responsible for the cure and prevention were known. Dumas, in 1871, noted that people fed on purified mineral, water, protein, fat and carbohydrate did not maintain good health. Lunin (1880) reported similar findings in animal experiments. Since addition of milk restored them to normal, he expressed the opinion that milk contained some 'unknown substances' required for maintenance of normal health. In 1897, Eijkman, a Dutch physician working in Java, observed that the disease beri-beri occurred mainly in people subsisting on a polished rice diet. He was able to produce polyneuritis in chicks and pigeons by feeding similar diets. Addition of rice polishings to the diet of human beri-beri patients cured them of the condition and similarly polyneuritis of chicks and pigeons also. Eijkman believed that the disease in the two cases was produced by a toxin present in polished rice and that this was neutralized by an antitoxin present in rice polishings. Grijns (1901) followed up these investigations and tried to isolate the toxin from polished rice, but he found none. He therefore suggested that there might be a protective substance in the rice polishings and that its absence in the diet caused beri-beri in human and polyneuritis in chick. Pekelharing of Holland, McCollum in America and Hopkins in England observed that symptoms produced by feeding purified protein, carbohydrate and lipid mixtures can be cured by adding whole milk or whey or even protein-freed milk to the diet (1911). The protective substances present in milk were named 'Accessory Factors' by Hopkins. In the same year Funk isolated from rice polishings a crystalline substance which could prevent or cure polyneuritis in pigeons. It was chemically an amine and was vital to life and hence he named it 'Vitamine'.

Further developments in the chemistry of vitamins have shown that only a few are amines. The term 'Vitamin' is retained now omitting the terminal 'e' in its spelling.

Several investigations were undertaken to evaluate the nutritive value of different types of food. Osborne and Mendel noted that butter fat, egg yolk or cod liver oil promoted growth of rats much better than when lard or almond oil formed the source of fat. Butter and cod liver oil also rapidly cured a condition of the eye which we now know as 'xeropthalmia'. McCollum named the factor present in these substances which was required for normal growth of animals as 'Fat-soluble A'.

Rickets is a disease known as early as 1650. It was also known that cod liver oil was useful in curing the condition. But it was only in 1918 that Mellanby produced rickets experimentally

in dogs and cured it by administering cod liver oil to such animals. Huldschinsky (1919) cured rickets in children by exposing them to ultraviolet light. McCollum and his associates (1922) showed that the curative agent from cod liver oil was different from vitamin A. Vitamin A activity was destroyed by heating to 100°C, but the antirachitic activity was preserved. Steenbock and associates and Hess independently showed in 1924 that antirachitic activity was acquired by certian foods when they were exposed to ultraviolet irradiations. Finally in 1931, Angus isolated crystalline Vitamin D_2 (calciferol) and Windaus (1936) isolated vitamin D_3. It was also McCollum who gave the name 'Watersoluble B' to the factor which was associated with milk sugar, which was water soluble and alcohol soluble and which was destroyed by heating. The absence of this factor was found to interfere with normal growth. Yeast was later found to be even better source for this 'Water soluble B' and in 1920 Emmett and Luros reported that autoclaving of yeast destroyed this factor which was anti-beri-beri but contained other substances that still promoted growth of rats on synthetic diets. Hence the 'Water soluble B' was shown to be composed of more than one factor. Thiamine was isolated in 1926 by Jansen and Donath and was synthesized in 1931 by Williams and associates.

With this fillip thus given to the vitamin research by these pioneers in the field, a number of individual workers and research teams identified and studied the several vitamins and even now the list of vitamins cannot be said to be complete. New factors having the role of vitamin are being reported from time to time.

Definition: Vitamins can be defined as naturally occurring organic substances which are required in minute amounts to maintain normal health of the organism and which have to be supplied in food as they cannot be synthesized by the organism. They supply very little energy by themselves, but they play an important role in several energy transformation reactions in the body. They differ from hormones in not being produced within the organism.

All vitamins are broadly divided into two groups — the fat soluble and the water soluble. To start with, they were given names according to the alphabet as vitamins A, B, C, D, E etc., before they were isolated in a pure state and their chemistry was known.

It will be advantageous to study the individual vitamins under certain general headings:

(a) Historical aspects
(b) Sources
(c) Chemistry
(d) Metabolism and physiological functions
(e) Deficiency diseases
(f) Assay methods
(g) Daily requirements and recommended dose
(h) Hypervitaminosis

Of these maximum stress will have to be laid on the study of the metabolism and physiological functions of the vitamins as the main purpose of their study in biochemistry.

FAT-SOLUBLE VITAMINS

Vitamin A

McCollum is credited with the discovery of this vitamin. He gave the name 'fat-soluble A to the substance.

Sources: Vitamin A occurs only in animal tissues but its precursors, carotenoid pigments, occur extensively in the vegetable kingdom. Cod liver oil and other fish liver oils, animal liver, milk and milk products and eggs all contain vitamin A. The carotenoid pigments are present in carrots, sweet potato, green vegetables like spinach and amaranth.

Chemistry: The vitamin has a characteristic ring structure called the *β*-ionone ring attached to a long hydrocarbon side chain ending in an alcohol group and is chemically known as 'retinol'. Its aldehyde form is known as 'retinal'. The provitamins, carotenes, have one or more beta ionone rings connected by a length of hydrocarbon chain. In case of *β*-carotene, the two rings at either end are *β*-ionone rings and on scission at the middle, it is capable of forming two vitamin A molecules (Fig. 9-1).

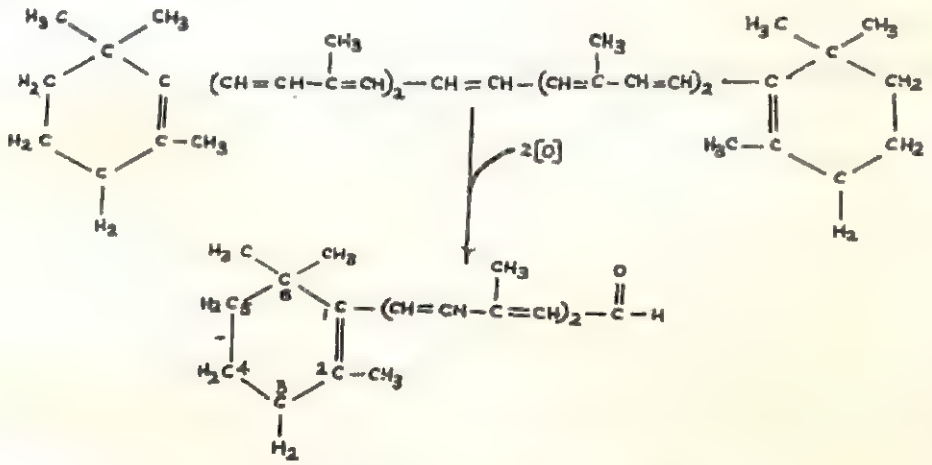


Fig. 9-1. *β*-Carotene and Vitamin A (aldehyde)

α and γ carotenes possess only one *β*-ionone ring and hence give rise only to one vitamin A molecule. The vitamin derived as above is called A_1 and is the most predominant form. It is the vitamin present in salt water fish livers. Fresh water fish livers contain Vitamin A_2 which has an additional double bond in the *β*-ionone ring.

Neo vitamin A is a stereoisomer of vitamin A_1. A_1 is the most active of all.

The vitamin is destroyed by oxidation and exposure to light.

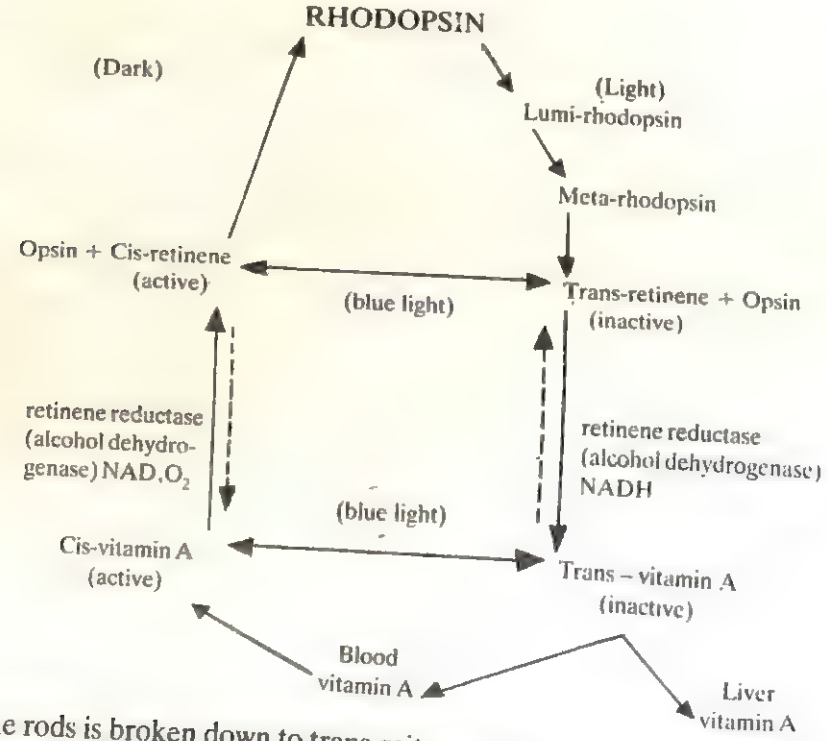
Metabolism and Physiological functions: The vitamin, as well as the provitamin, are readily absorbed by the intestines along with lipids. The vitamin is present as fatty acid ester in natural sources. It has to be liberated in the free form by digestion by pancreatic esterase

before it can be absorbed. It is re-esterified in the intestinal mucosa before absorption into the lymphatics. Carotenes are converted to vitamin A by the intestinal epithelium in some species and by the liver in man. Good amounts of the vitamin can be stored in the mammalian liver also, with the result that symptoms of deficiency occur only after a long period of vitamin deprivation from diet.

Retinol is esterified with fatty acids for storage in the liver. For visual function, it is the aldehyde form (retinal or retinene) that is required, whereas for growth and epithelial functions, its oxidation product, retinoic acid, is required.

The conversion of carotene to vitamin A does not occur efficiently in diabetes mellitus and hypothyroidism. They will therefore develop deficiency symptoms if vegetables are the only source of the vitamin.

Role in visual function: The rods of the retina contain a pigment called 'rhodopsin' or 'visual purple' which is concerned with vision in dim light. The pigment is a conjugated protein–a protein component opsin and prosthetic group of reitnene (which is vitamin A aldehyde). The cones contain three different pigments, porphyropsin, iodopsin and cyanopsin, which are sensitive to the three colors–red, green and blue respectively. All the three pigments are conjugated proteins (opsins) with retinal as the prosthetic group.



Rhodopsin of the rods is broken down to trans-reitnene and opsin on exposure to light. The trans-retinene can be converted to cis-retinene in vitro in the presence of blue light and can then recombine with opsin to form rhodopsin. But, in vivo, this does not occur. The alcohol

dehydrogenase enzyme of retina rapidly reduces trans-retinene to trans-vitamin A, which enters the blood stream and is carried to the liver where it is converted to its cis isomer. The cis vitamin A thus formed in liver enters circulation again and reaches the retina where it is then oxidized to cis-retinene and is now able to form rhodopsin with opsin.

Thus vitamin A is concerned very much with rod-vision (dim-vision) and its deficiency causes a condition known as night blindness (nyctalopia) i.e. inability to adapt when suddenly proceeding from bright light to dim light.

Ca^{++} ions are required for the normal functioning of the visual cycle.

Zinc is necessary to maintain normal plasma concentration of vitamin A. Patients suffering from cirrhosis of the liver have a low concentration of zinc in plasma and impairment of dark adaptation. Therapy with vitamin A does not improve them.

General Metabolism: Vitamin A seems to be required in the conversion of triose molecules to glucose. Whether it is a direct effect or an indirect one through the adrenal cortex is not clear (the synthesis of cortical hormones which are mainly concerned in gluconeogenesis might be inhibited in vitamin A deficiency).

In vitamin A deficiency, there is impairment of oxidative phosphorylation in the liver mitochondria. Excessive amounts of the vitamin are required for the normal mitochondrial membrane function.

Growth and reproduction: Growth as well as reproductive functions are impaired in rats kept on vitamin A deficient diets.

Epithelium: The epithelial structures of skin and mucous membrane show gross structural and functional changes in its deficiency. Skin becomes dry, scaly and rough–changes described as keratinization. Similar changes occur in lacrimal glands leading to dryness of conjunctiva and cornea (xeropthalmia). White opaque spots (Bitot's spots) appear in the conjunctiva on either side of the cornea in each eye. Corneal epithelium also becomes keratinized and opaque and may become softened and ulcerated. The condition is called keratomalacia. Keratinization occurring in the mucous membrane of the respiratory tract leads to increased susceptibility to infection and lowered resistance to disease. Vitamin A is also called anti-infective vitamin on account of its ability to prevent infection. Keratinization of urinary tract may lead to formation of calculi.

The skin lesions do not occur if adequate amounts of B-complex vitamins are available. A simultaneous deficiency alone will cause the typical lesions.

Retinol is concerned in the synthesis of chondroitin sulfate, probably by stimulating the formation of active sulfate or by preventing its destruction.

Bones and Teeth: Deficiency of the vitamin may result in lowered osteoblastic activity. Bones become cancellous. Osteoclastic activity is also impaired leading to defective resorption of bone. This results in diminished intracranial capacity, compression of the brain and also cranial and spinal nerves. Teeth also become unhealthy due to thinning of enamel and chalky deposits on the surface. All the above deficiency symptoms of experimental animals occur to a varying extent in deficiency states in the human being also.

Assay: Bioassay can be made by measuring the growth rate of rats deprived of other sources of the vitamin and maintained on graded amounts of the vitamin. The vitamin activity contained in 0.6 μg of pure β carotene or 0.3 μg of pure vitamin A_1 is known as an international unit (I.U.), or a U.S.P. unit. It can be also measured colorimetrically by measuring the blue color developed in chloroform solution with antimony trichloride.

Requirements: The conversion of beta carotene to vitamin A is only 50% effective, i.e. instead of two molecules of vitamin for each molecule of beta carotene, only one molecule is produced. (Hence the international unit of beta carotene is 0.6 microgram while that of vitamin A is 0.3 microgram). Further, the absorptin of carotene is only about one third of the amount present in food. Hence, if carotene is the main source of the vitamin, much larger amounts have to be provided. The FAO/WHO expert group (1967) recommended a daily intake of 750 microgram (2,500 I.U.) of vitamin A or 3,000 microgram (5,000 I.U.) of beta carotene for an adult. The Nutrition Expert Group, India (1968) also recommended the same intake. Pregnant and lactating women have to be provided 50% more.

Hypervitaminosis: This is one of the few vitamins, which, if taken in excessive doses over long periods, produces toxic symptoms. Headache, nausea, vomiting, drowsiness, loss of appetite and pains in bones are some of the symptoms observed.

Vitamin D

The credit for demonstrating the antirachitic activity of cod liver oil as residing in a factor other than vitamin A goes to McCollum. Subsequently the vitamin was isolated in crystalline form by Angus, Windaus and others. Vitamin A is destroyed by oxidation readily, while vitamin D is not.

Two main forms of the vitamin were discovered–(1) Calciferol or vitamin D_2 obtained by irradiating (with ultravoilet light) the plant sterol ergosterol and (2) vitamin D_3 formed by irradiating the animal sterol, 7-dehydrocholesterol. Dihydrotachysterol is also produced during irradiation of ergosterol and it is called AT-10. This has also the vitamin activity in some respects. Other derivatives including vitamin D_1 are not sufficiently active.

Sources: Egg yolk, milk, butter and fish liver oils contain varying amounts of the preformed vitamin, the highest amounts being present in fish liver oils.

Chemistry: The structure of calciferol is shown in Fig. 9-2.

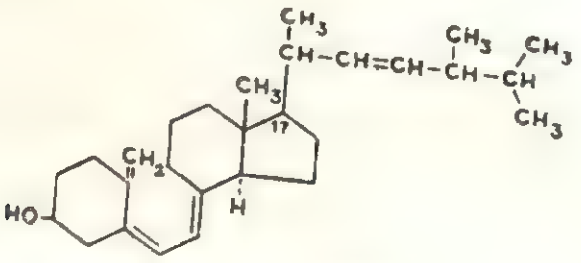


Fig. 9-2. Calciferol

They differ from the provitamins ergosterol and 7-dehydrocholesterol only in the B ring which has opened out during the activation process and the methyl group at position 10 is replaced by a methylene group.

Metabolism and Physiological Functions: The vitamin (either D_2 or D_3) is readily absorbed from gastro-intestinal tract along with fats. It is also absorbed by the skin if, say, cod liver oil is massaged on the skin. Liver, skin and even brain, lungs and bones store the vitamin. It is secreted in milk and bile. Urinary excretion is practically nil. Part of the vitamin is broken down slowly in the body to as yet unidentified substances.

The main function of the vitamin is in relation to calcium and phosphorus metabolism and mineralization of the skeleton.

The active form of the vitamin appears to be a dihydroxy derivative. One –OH is added at C-25 by the liver. The kidney adds one more –OH at C-1. The addition of –OH by the kidney is regulated by the serum calcium level — low levels accentuating the function, while normal or high levels inhibit the reaction (an example of feed-back inhibition) . In chronic renal disease, deficiency of the hydroxylating mechanism may be one reason for low calcium levels in serum and vitamin D-resistance of the condition.

1. Vitamin D promotes abosrption of calcium and phosphorus by the intestines. It stimulates the synthesis of a specific calcium binding protein by the intestinal epithelium. The protein participates in the active transport of calcium across the small intestinal mucosa.

2. It promotes growth in general. This is probably dependent on the action of the vitamin in growth of long bones.

3. It facilitates the normal functioning of parathormone.

4. It promotes mineralization of bones through its action on calcium and phosphorus metabolism and also by a direct action on the bone tissue. It facilitates the action of parathormone in the above functions and increases renal excretion of phosphate.

5. AT-10 has a powerful hypercalcemic effect on oral administration, resembling parathormone in this respect. It is sometimes used in the treatment of tetany. That is how it derived its name — anti-tetany compound, number 10-abbreviated as AT-10. Like parathormone, it also increases the excretion of phosphorous in urine.

6. It somehow acidifies the pH of distal illeum, colon and cecum.

7. It increases citrate content of bone, blood and other tissues.

   Many of these actions are probably by enhancing transport of calcium across the cell membrane — be it the intestinal epithelium or bone. The transport may be enzyme mediated.

8. It exerts an anti-rachitic effect. Rickets is a disease involving bone growth.

Normally growth occurs at the ends of long bones by a proliferation of the bone forming cells, osteoblasts, which invade the epiphyseal cartilage. The cartilage cells degenerate and disappear. Capillaries grow into the site and the bone matrix is laid down by the osteoblasts around the invading capillaries. Finally mineralization i.e. deposition of calcium and phosphorus salt takes place. The whole process is continuous and bone continues to be laid in the receding cartilage. The calcium and phosphorus salts ultimately form what is known as hydroxyapatite. An enzyme vitally concerned in the precipitation of calcium and phosphorus is alkaline phosphatase. It hydrolyzes organic phosphate esters and liberates phosphate. This increases the Ca x P product. Normally this is 40 (Ca x P = 10 x 4) and is the ideal concentration to keep both minerals in solution. When Ca x P product is exceeded due to liberation of excess phosphate, both are precipitated. Vitamin D seems to exert a stimulating action on bone alkaline phosphatase. It may also exert an effect on the activity of the osteoblasts themselves.

Deficiency disease: Rickets in growing children and osteomalacia in adults are produced by a deficiency of vitamin D.

Rickets: Instead of growth occurring normally at the end of long bones as described above, the osteoblast proliferation does not take place in an orderly fashion and is not accompanied by vascularization or mineralization at the normal rate. This results in irregularity in the zone of provisional calcification. The cartilage cells do not degenerate as they should and the ends of the long bones bcome bulky and soft. The bone mineral may be reabsorbed away from the shaft of the long bone making it soft. Bending of the long bones giving rise to deformities such as bow-legs and knock knees occur when the child attempts to stand up and walk. The ankles, knees, wrists and elbows are swollen due to swelling of epiphyseal cartilages. The fontanelles do not close properly (hot cross bun appearance of the head). The ribs present a beaded appearance (rickety rosary) at the costo-chondral junctions. The chest gives a pigeon-breast appearance. Teeth erupt late and are deformed.

Osteomalacia: The deficiency of the vitamin in the adult is rare on account of the ability of the skin to synthesize it from 7-dehydrocholesterol (available in the skin) in the presence of sunshine which provides the ultraviolet irradiation necessary. However, in conditions like (i) pregnancy and lactation where there is extra demand for the vitamin and drain of the vitamin due to secretion in milk, and (ii) in women observing purdah or in climates where sunshine is scanty, a deficiency of the vitamin may develop and cause a condition known as osteomalacia (which literally means softening of bones). The formed bones get demineralized and soft. This particularly affects the pelvic bones which incidentally bear the brunt of the stress in pregnancy. They become deformed and further complicate parturition (child birth).

In both the cases, the serum calcium tends to be below normal (normal is 9-11 mg/100 ml). Urinary excretion of phosphorus is increased due to diminished tubular reabsorption and a gross increase in serum alkaline phosphatase is also noted.

Assay methods: Colorimetric methods are available. But more useful are the biological methods wherein a deficiency is produced artificially in rats or chicks and (i) the amount of

the substance required to heal the rickets in rats (as evidenced by studying the line of provisional calcification in long bones by postmortem (or by X-ray) or (ii) the amount required to maintain the calcium content (ash) of the bones in the chick is measured. In general, rats react quicker to D_2 and chicks to D_3. An International Unit (I.U.) or U.S.P. unit is defined as the activity present in 0.025 μ g of crystalline D_3.

Daily requirements: The National Research Council (U.S.A., 1964) recommended intake of 400 I.U. per day for infants and growing children. In tropical countries with plenty of sunlight, smaller amounts may suffice. The Nutritional Expert Group, India, recommended a daily supply of 200 I.U.

Hypervitaminosis: Extremely large doses produce hypercalcemia, hyperphosphatemia, anoerxia, nausea, vomiting and diarrhoea. Metastatic calcification may occur in kidneys, arteries, bronchi and other sites.

Vitamin E

The E group of vitamins are derivatives of tocopherols. It was Mattill and Conklin in 1920 who first reported the interrelation of food factors with reproduction. In 1922 Bishop and Evans termed the food factor as factor 'X' which was subsequently renamed vitamin E.

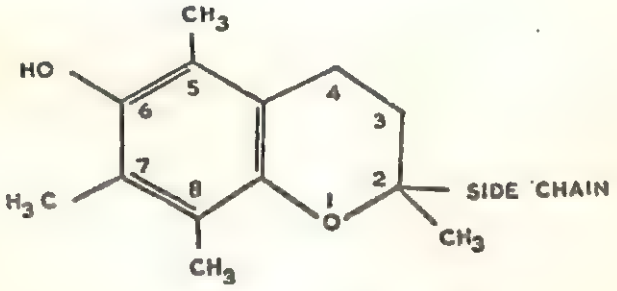


Fig. 9-3. Tocopherol

Chemistry: Several compounds belonging to the group of tocopherols exhibit vitamin activity. The name tocopherol is derived from the fact that the vitamin is concerned with normal child birth (tokos = child birth; phero = to bear; 'ol' stands for alcohol). The structure is shown in Fig. 9-3.

The double ring structure is a 'chromane' ring.

The 5,7,8 trimethl derivative is called α tocopherol and has the highest vitamin activity. Other derivatives (β, γ etc.) are derived by altering the position of the $-CH_3$ groups. They are oily substances and are stable to heat. They are readily oxidized and act as powerful antioxidants and protect other vitamins like A from oxidation when present with them.

Sources: Vegetable oils, particularly wheat germ oil, are rich in the vitamin content. Corn oil, cotton seed oil and safflower oil are good sources.

It is also present in fair amounts in egg-yolk and green leafy vegetables like spinach and lettuce.

Metabolism, functions and deficiency symptoms: It is readily absorbed with fats from the gastrointestinal tract and is metabolized to unidentified substances in the tissues. Vitamin E as such is not excreted to any extent.

The antioxidant action is exerted by the vitamin in vivo also with the result it minimizes the need for vitamin A. Deficiency of the vitamin in the rat and chick causes a brownish discoloration of the adipose tissue and a yellowish discoloration of the enamel of the teeth due to oxidation of the unsaturated fatty acids present in these structures to peroxides. The requirement for the vitamin seems to depend on the amount of polyunsaturated fatty acids in the diet. Low vitamin E containing diets in malnourished children are shown to produce a macrocytic anemia, increased fragility of the R.B.C., thrombocytosis and edema-all of which are relieved by addition of vitamin E to the diet.

Vitamin E exerts a protective action in preventing massive hepatic necrosis produced on diets deficient in sulfur containing amino acids. In this function it resembles the protective action exerted by cysteine and selenium compounds. Its specific functions, however, relate to its role in reproductive physiology. In male rats deprived the vitamin, the seminiferous epithelium undergoes irreversible degeneration and permanent sterility occurs. In female rats, the deficiency does not affect the ovary. Estrus, ovulation, conception and implantation take place normally. But the fetus dies in utero a few weeks after conception and undergoes resorption. If the vitamin is administered within ten days of conception, the fetus survives and normal delivery takes place.

There is variation from species to species in the action on reproduction. In the human being, no definite role of the vitamin is seen in either the causation or prevention of abortion or sterility.

Another effect of the deficiency of the vitamin in experimental animals is muscle dystrophy, a degenerative disease of sekeletal muscle. Creatine of muscle passes out into the blood increasing thereby the blood and urine creatine. The condition is cured by vitamin E administration. Man also suffers from muscular dystrophy, but it is not produced by vitamin E deficiency and is not cured by its administration.

A derivative of the vitamin is said to be necessary for the synthesis of conezyme Q which is a component in the electron transport system.

Assay: Bioassay methods measure the amount of the substance required to support gestation in a vitamin deprived pregnant rat. Chemical methods are available.

Requirements: Average human diets contain about 30 I.U. (20 mg.) of D-alfatocopherol and since no deficiency is ever reported, this is considered adequate. Hypervitaminosis symptoms are not reported.

Vitamin K

Chicks fed on synthetic rations developed a hemorrhagic disease. Dam (1935) named the factor present in natural diets and which protected against the hemorrhagic disease as vitamin K (Koagulation vitamin). Dam and Karrer in 1939 isolated the vitamin in crystalline form.

Chemistry: It has a naphthoquinone structure and is closely related to the compound known as pthiocol. Vitamin K_1, K_2, and K_3 are the important derivatives. The structure of pthiocol is shown in Fig. 9-4.

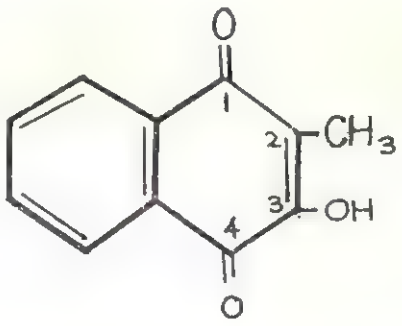


Fig. 9-4 Pthiocol (2-Methyl-3-Hydroxy-I, 4-Napthoquinone)

If OH at position 3 is replaced by a phytyl side chain, it is vitamin K_1. This is present in vegetable sources. If it is replaced by a difarnesyl side chain, it is vitamin K_2. This is produced by bacterial synthesis. If the OH at position 3 is removed without any other substitution, it is vitamin K_3, also known as menadione. Menadione is particularly important on account of its water solubility. It can be readily administered by the parenteral route.

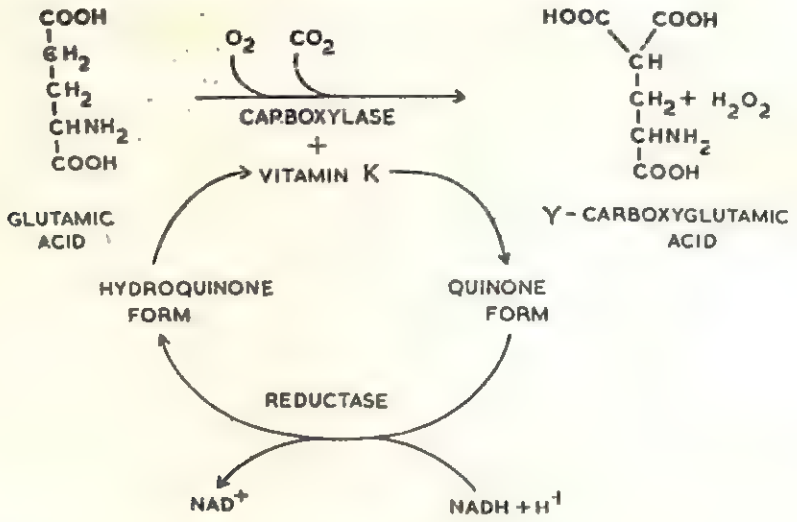
Sources: Vitamin K occurs extensively in green leafy vegetables such as spinach, alfalfa grass, cauliflower, cabbage and tomato. It also occurs in putrid fish meal in large amounts. It can be synthesized by microorganisms inhabiting the gastrointestinal tract of higher animals including man and the vitamin so synthesized is absorbed and utilized by the animal.

Metabolism and functions: It is readily absorbed along with fats in the presence of bile salts. It is not stored to any appreciable extent. Hence a constant supply is needed. It can be transferred to the fetus in utero through the placenta. It is also secreted in milk. Feces contains large amounts of the vitamin. This is of bacterial origin. No excretion occurs through urine.

Vitamin K is required for the formation of a number of coagulation factors in the liver- proconvertin, plasma thromboplastin component (PTC, factor IX), Stuart factor (factor X) and prothrombin. The deficiency of the vitamin is reflected by the overall effect of an increase in the prothrombin time.

Prothrombin, Factors VII, IX and X are all synthesized by the liver in an inactive form. They are activated by converting some of the glutamic residues (Glu) to γ -carboxyglutamic acid (Gla). This conversion requires vitamin K. The Gla residues help to chelate calcium in

a protein-calcium-phospholipid interaction. A similar reaction occurring in an acidic protein *osteocalcin* present in the bone matrix helps in the mineralization of bone.



It is also said to participate in the phosphorylation reactions of photosynthesis in plants and has probably a similar role in the electron transport system in animal tissues as well. A quinone which is widely distributed in animal tissues (hence called ubiquinone) acts as a coenzyme and is named coenzyme Q. It has a role in electron transport between the flavin coenzymes and the cytochromes. Its structure is as shown in Fig. 9-5.

The structural similarity between this and the vitamin K suggests a coenzyme function for the vitamin.

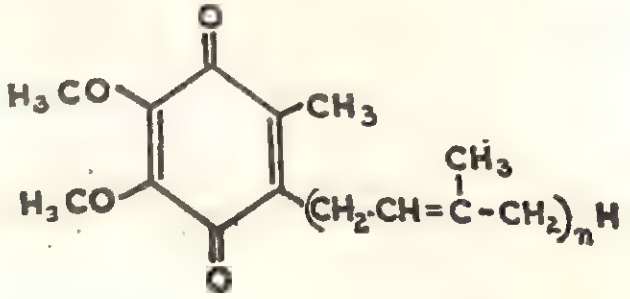


Fig. 9-5. Coenzyme Q

Deficiency: A deficiency of the vitamin does not normally occur on account of its availability in plenty from intestinal bacterial synthesis. However in new born infants (particularly premature infants) where the intestinal flora have not yet established themselves, a deficiency may occur. This can be prevented by injecting the vitamin into the mother just before child birth so that it can be transferred intraplacentally to the child. A deficiency also occurs when drugs like sulfonamides and antibiotics are administered. They kill the intestinal

microorganisms and cut off an important source of vitamin supply. Obstructive jaundice (by causing absence of bile salts in the intestines and thus impairing absorption of fats and fat-soluble vitamins) and other malabsorption conditions also produce a deficiency of the vitamin. The manifestation in all these cases is a delayed prothrombin time and a hemorrhagic tendency.

Requirements: On account of intestinal synthesis, the vitamin need not be supplied normally. But where this is interfered with, 1-2 mg of menadione has to be supplied.

Vitamin K antagonists: A substance called dicumarol present in sweet clover hay is known to produce a hemorrhagic disease in cattle when they ingest the hay. This is on account of the structural similarity of dicumarol to vitamin K whereby it acts as a competitive inhibitor for the enzymes concerned with synthesis of prothrombin and other coagulation factors that require vitamin K probably as a coenzyme. The structure of dicumarol may be seen in Fig. 9-6.

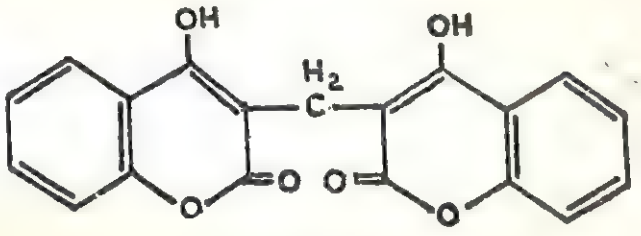


Fig. 9-6. Dicumarol

WATER SOLUBLE VITAMINS

Vitamins of the B complex group and vitamin C belong to this group. As the name indicates they are all water soluble.

B Complex group of vitamins

The anti beri-beri factor present in rice polishings, yeast and liver was originally called the 'water soluble vitamin-B' to distinguish it from the 'fat-soluble-A' known at the time (in the early 1920's). In course of time several water soluble factors acting as vitamins were found to be present in the same sources — rice polishings, yeast and liver — and these were named B_1, B_2 etc. Subsequently their chemical structure was identified and they were assigned more rational names based on their chemistry. In general they all have well defined coenzyme functions in the cellular metabolism unlike the rather vaguely understood metabolic functions of their fat soluble brethren. Some of them are synthesized in the tissues of the higher animals, and so do not strictly satisfy the definition of 'vitamin', but are included in this group on account of the close functional relationship to the other members of the group. The factors included in this group are: (1) Thiamine (B_1), (2) Riboflavin (B_2), (3) Niacin, (4) Pantothenic acid, (5) Pyridoxine (B_6), (6) Biotin, (7) Folic acid, (8) Cobalamine (B_{12}), (9) Lipoic acid, (10) Inositol, (11) Choline and (12) Para-aminobenzoic acid (PABA).

Sources: Generally they are rich in germinating seeds, rice polishings, wheat germ, pulses, beans and lentils, yeast, liver and meat.

Assay: Chemical methods of assay include colorimetric, spectrophotometric and photofluorimetric methods which all depend on extracting the vitamin from the material into suitable solvents and treating the extracts with suitable reagents to develop a color or fluorescence which can be measured using a colorimeter, spectrophotometer or photofluorimeter.

2. Bioassay methods include methods whereby a deficiency condition is produced in a group of susceptible animals and the amount of the substance required to cure the condition by well defined criteria is compared to the amount of pure crystalline vitamin standard.

3. Microbiological assay: Instead of employing experimental animals whose feeding and maintenance is expensive, certain microorganisms whose growth requires the vitamin in question may be used. The microorganisms are cultured on agar plates or nutrient broth solutions which contain all other nutrients except the vitamin which has to be assayed. The unknown substance is added to some cultures and measured amounts of the purified vitamin to others. The growth in the unknown is compared to that in the standards. This can be readily done by measuring the number and size of the colonies on the agar plate or by preparing a suspension of the growth of the organisms in saline and measuring its turbidity.

Anti-vitamins: In the case of a number of vitamins in this group, there is marked antagonism exhibited by structurally similar substances which are named anit-vitamins. These are examples of competitive inhibition leading to blocking of the specific enzyme functions. Anti-vitamins, when present, will induce vitamin deficiency symptoms which can be countered only by administering unusually large doses of the vitamins.

Synthesis: Many of the vitamins of the group can be synthesized in the laboratory and are available in a pure crystalline form.

Loss in cooking process: Washing the material before cooking and draining the water after cooking will result in loss of much of the water soluble vitamins.

Thiamine

(Synonyms: Vitamin B_1; anti-beri-beri substance; antineuritic vitamin; aneurine).

The history of the discovery of this vitamin was already mentioned.

Chemistry: Thiamine is a basic substance and contains a pyrimidine ring linked to a thiazole ring. It can be isolated in a crystalline form as the chloride-hydrochloride. It is fairly stable in acid solutions but is rapidly destroyed in alkaline medium. Its structure is shown in Fig. 9-7.

The –OH of the hydroxyethyl group in the thiazole can be esterified with 2 molecules of phosphoric acid to form thiamine pyrophosphate (TPP) which is the active coenzyme form of the vitamin.

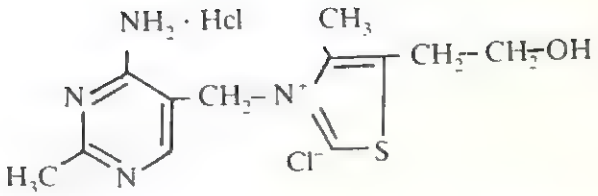


Fig. 9-7. Thiamine Chloride (2, 5-Dimethyl-6-aminopyrimidine +4-Methyl, 5-Hydroxyethyl Thiazole)

Sources: As for B complex in general.

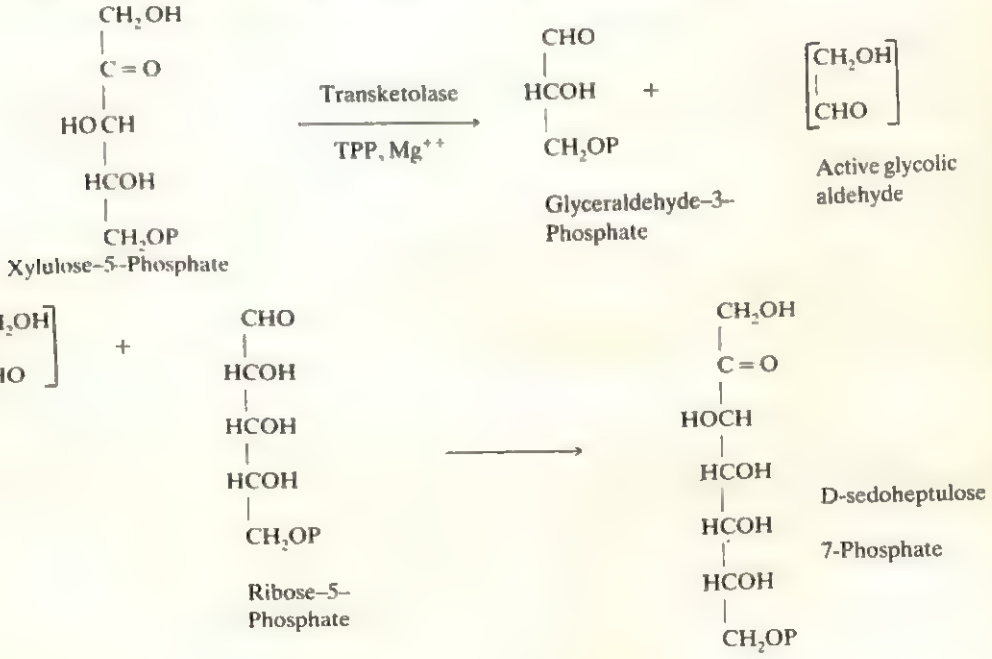
Assay: Oxidation will yield thiochrome which can be assayed by its fluorescence. Biological and microbiological procedures are available.

Metabolism and functions: Free thiamine is readily absorbed by the small bowel. It is phosphorylated mainly in the liver to form TPP. Varying amounts of the coenzyme are present in all tissues. But there is no storage of the vitamin. Hence regular supplies are needed in the diet. About 10% of the ingested vitamin is normally excreted in urine.

TPP acts as coenzyme in oxidative decarboxylation reactions. Mg^{++} ions are required as activators.

$CH_3CO\,COOH$ (Pyruvic acid) $\xrightarrow[\text{(Pyruvic acid dehydrogenase)}]{\text{TPP, } Mg^{++}}$ $CH_3CHO + CO_2$ (acetaldehyde)

The decarboxylation of alfa ketoglutaric acid is similar. It also acts as coenzyme in the transketolation reactions in the HMP pathway of glucose metabolism.



TPP is also required as coenzyme for the oxidative decarboxylation of branched chain amino acids — valine, leucine and isoleucine. The enzyme concerned is *branched chain* α -ketoacid dehydrogenase. FAD, lipoic acid and NAD are also required along with Mg^{++} ions.

Deficiency: The deficiency of the vitamin produces a condition called beri-beri. This has essentially two components *(i)* cardiovascular failure leading to enlargement of the heart and edema and *(ii)* neural component, viz. peripheral neuritis. A deficiency in pigeons produces a disease characterized by loss of appetite, paralysis and head retraction (opisthotonus).

The pyruvate and alfa-ketoglutarate levels of blood are increased and the activity of the enzymes, pyruvate dehydrogenase and alfaketoglutarate dehydrogenase, is decreased. Erythrocyte transketolase levels are also lower than normal.

The blood-brain barrier is impermeable to pyruvate normally in rats, but becomes permeable in thiamine deficiency. This will naturally necessitate alteration of metabolic pathways in the brain.

Thiamine antagonists: Pyrithiamine (where the thiazole ring is replaced by a pyridine ring) is a potent antagonist to thiamine. Oxythiamine and 2-n-butyl thiamine are milder antagonists. Foxes develop a type of paralysis called Chastek paralysis when they eat raw fish. This is on account of the presence of an enzyme 'thiaminase' in raw fish which destroys thiamine.

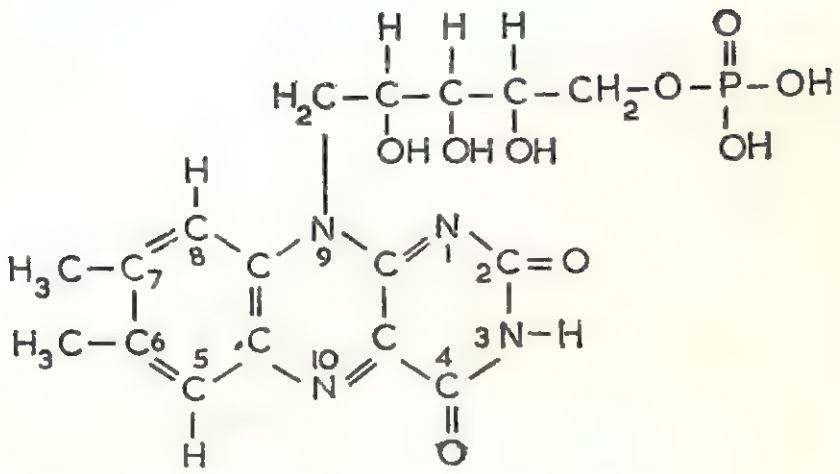
Requirements: They depend mainly on the caloric intake and particularly carbohydrate intake of the individual. For an adult taking 3,000 calories per day, 1.5 mg. of thiamine are required.

Riboflavin

(Synonyms: Vitamin B_2, Lactoflavin)

The vitamin was isolated from several sources by different people and named according to source as lactoflavin (from milk), ovoflavin (from egg-yolk) and hepato-flavin (from liver). Warburg and Christian in 1932 isolated the yellow respiratory pigment (Warburg's yellow enzyme) from bottom yeast. Subsequently the pure vitamin was isolated and synthesized.

Chemistry: Riboflavin is derived from a parent ring structure called isoalloxazine. Ribityl alcohol is combined with a substituted isoalloxazine to form the riboflavin (See Fig. 9-8).



6, 7-Dimethyl-9 (D-ribityl-5-phosphate)-isoalloxazine
Fig. 9-8. Riboflavin

The ribityl alcoholic group can be esterified with phosphoric acid to form riboflavin phosphate or flavin mononucleotide (FMN). It may be linked to adenine nucleotide through a pyrophosphate linkage to form flavin adenine dinucleotide (FAD). (Flavin-ribityl-P-P-ribose-adenine). FMN and FAD are the two coenzyme forms of the vitamin.

Sources: Milk, liver, kidney, heart, egg yolk and germinating seeds.

Riboflavin is destroyed on exposure to light and is reduced to colorless products.

Functions: FMN as well as FAD act as coenzymes in various hydrogen transfer reactions in metabolism. The hydrogen is transported by reversible reduction of the coenzyme by 2 hydrogen atoms added to the 'N' at positions 1 and 10. The enzyme reactions catalyzed are as follows:

FMN	*FAD*
Warburg's yellow enzyme	Xanthine oxidase
Cytochrome Creductase	D-amino acid oxidase
L-amino acid oxidase	Aldehyde oxidase
	Succinate dehydrogenase

Deficiency: Lesions of lips, fissures at angles of the mouth (cheilosis), seborrheic dermatitis of face, glossitis (magenta red tongue) and vascularization of conjuctiva and cornea are observed in its deficiency.

Deficiency produces cataracts in some experimental animals (rats).

Assay: Microbiological assay uses L-casei (Lactobacillus casei) wherein the production of lactic acid by the organisms is measured. Other general methods are also applicable.

Requirements: 1.5 to 2.0 mg. per day. Nutritional Expert Group, India, has recommended an intake of 0.55 mg. per 1,000 calories, same as that recommended by FAO/WHO group.

Antagonists: Dichlororiboflavin (chlorine replaces the methyl groups in positions 6 and 7) and isoriboflavine (the methyl groups are shifted to positions other than 6 and 7) act as antivitamins.

Niacin

(Synonyms: P-P-factor; pellagra preventing factor of Goldberger; Nicotinic acid).

Goldberger (1912) identified pellagra as a disease caused by deficiency of a dietary factor. 'Canine black tongue' in dogs is a similar deficiency disease. Elevehjem in 1937 isolated nicotinic acid and its amide from liver extract and showed its efficiency in curing these conditions.

Chemistry: Niacin is pyridine-3-carboxylic acid. Its amide is niacinamide. Its biologically active forms are (1) a dinucleotide — niacinamide-adenine-dinucleotide (NAD) and (2) its phosphorylated form — niacinamide-adenine-dinucleotide phosphate (NADP). The structure of these compounds may be seen in Fig. 9-9.

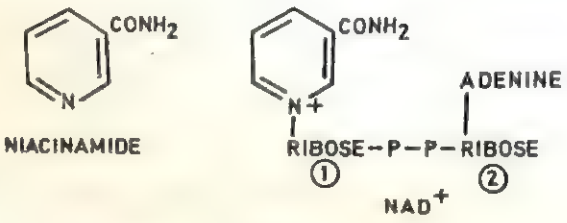


Fig. 9-9. NAD

If a phosphate is added to ribose (2), it becomes NADP.

Sources: The general sources of B complex contain this vitamin also.

Assay: Microbiological methods employ estimation of acid produced by lactobacillus arabinosus. Chemical methods use color development with cyanogen bromide.

Metabolism and functions: It can be synthesized from tryptophan, the essential amino acid (see under tryptophan metabolism). 60 mg. of the amino acid approximately supply 1 mg. of niacin. It is readily absorbed by the intestine. It is excreted in urine after methylation of the 'N' of the pyridine ring.

NAD and NADP function as coenzymes for hydrogen transfer enzymes (dehydrogenases). Two hydrogen atoms are transported; one atom is taken up in the pyridine ring and an electron from the second atom is taken up by the + vely charged pyridine nitrogen, leaving hydrogen ion in the medium (See Fig. 9-10).

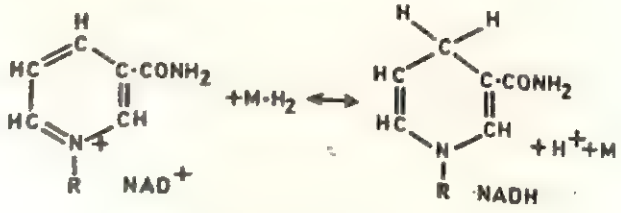


Fig. 9-10. NAD^+ and $NADH + H^+$

Some of the reactions where the two coenzymes are used are listed below:-

NAD	*NADP*
Alcohol dehydrogenase	Isocitric acid dehydrogenase
Lactic acid dehydrogenase	Glucose-6-phosphate dehydro-genase
Malic dehydrogenase	
Phosphoglyceraldehyde dehydrogenase	

Either

Glutamic acid dehydrogenase

Deficiency: The disease called pellagra (pelle = skin; agra = rough) is produced on account of the deficiency of niacin. It is characterized by dermatitis, diarrhoea and dementia (3 'D's) and if not treated the 4th 'D' death follows.

The dermatitis involves extensor surfaces of forearms, the exposed V of the neck, perinium and other areas exposed to Sun or pressure. Loss of appetite, vomiting, diarrhoea, stomatitis and soreness of tongue are the gastrointestinal symptoms. Mental depression, anxiety and other psychosis may occur.

Requirements: National Research Council, U.S.A. recommended 6.6 mg. per 1,000 calories (FAO/WHO group). This was also confirmed by the Nutrition Expert Group for Indian conditions. For a 3,000 calorie diet, this works out to 20mg/day.

Pharmacological actions: Niacin (but not niacinamide) causes a transient but marked vasodilatation with flushing and sensation of warmth in the face, neck and arms. Blood flow to the skin increases The plasma lipid concentration is decreased. Niacin is therefore used in the treatment of hyperlipemia.

Pyridoxine

(Synonyms: Vitamin B_6: rat antidermatitis factor; rat acrodynia factor; adermin; vitamin H).

Gyorgy demonstrated that a characteristic dermatitis of the rat required a factor different from the three known vitamins discussed earlier (thiamine, riboflavin, and niacin). It was present

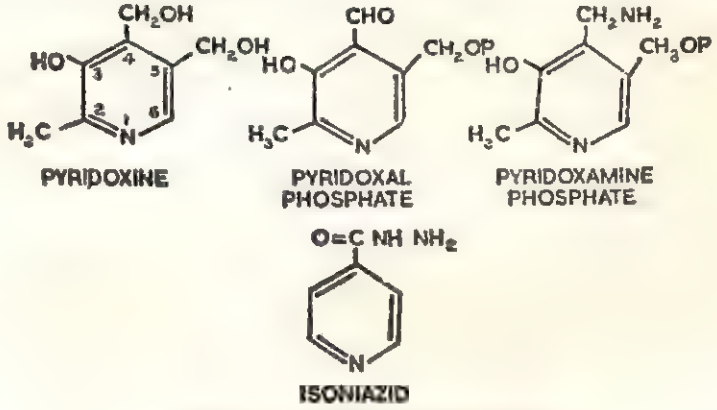


Fig. 9-11. Pyridoxine and derivatives.

in yeast extracts and could be adsorbed on to charcoal. He called it the vitamin B_6. Its chemical nature was elucidated subsequently and it is now commonly known as pyridoxine.

Chemistry: Pyridoxine is 2-methyl-3-hydroxy-4, 5-di (hydroxymethyl) pyridine. The active form of the vitamin is pyridoxal phosphate in which the $-CH_2OH$ at 4 is converted to a $-CHO$ group (removal of 2H) and the $-CH_2OH$ at 5 is esterified with phosphoric acid. The aldehyde group at 4 can reversibly combine with NH_3 to form pyridoxamine phosphate.

The structures of all these are given in Fig. 9-11.

Sources: Same as other members of B-complex.

Metabolism and functions: The vitamin present in food is readily absorbed by the intestines. There is no appreciable storage of the vitamin. Most of it is excreted in urine after oxidation of the 'CHO' in position 4 to 'COOH' to form pyridoxic acid.

1. The vitamin is mainly concerned in the metabolism of amino acids. Its ability to take up ammonia reversibly to form pyridoxamine phosphate enables it to act as a coenzyme in transamination reactions (see under coenzymes).

2. It also functions as a coenzyme in nonoxidative decarboxylation of some amino acids or their derivatives.

   e.g. 3,4-dihydroxyphenylalanine $\longrightarrow$ DOPAMINE + CO_2
   Glutamic acid $\longrightarrow$ Gamma aminobutyric acid (GABA) + CO_2
   Tyrosine $\longrightarrow$ Tyramine + CO_2

3. It is necessary for the conversion of tryptophan to niacin. It acts as a coenzyme in the step involving conversion of 3-hydroxykynurenin to 3-hydroxyanthranilic acid.

4. It is also required in the interconversion of glycine and serine where it functions along with folic acid.

5. It takes part in transsulfuration reactions involving transfer of –SH groups e.g. homocysteine + serine $\longrightarrow$ homoserine + cysteine.

6. It is also required for synthesis of sphingosine from serine and arachidonic acid from linoleic acid.

7. A general role of facilitating the transport of amino acids and some cations across the cell membrane is ascribed to this vitamin.

Deficiency: Epileptiform convulsions in human infants are said to be due to the deficiency o the vitamin. But there is no conclusive evidence and therapeutic use of the vitamin in the condition is of doubtful efficacy. A hypochromic, microcytic anemia may develop in its deficiency. (The vitamin as a decarboxylase is required at one stage in heme synthesis).

Homocystinuria and cystathioninuria occur in pyridoxine deficiency due to impaired metabolism of methionine.

Treatment with isoniazid (isonicotinic acid hydrazide) (INH) in tuberculous patients induces a B_6 deficiency state manifested by neuropathy and excretion of abnormal metabolites of tryptophan in urine. This is said to be on account of a complex formation between INH and pyridoxal, thus blocking normal functioning of the latter. Large doses of the vitamin will overcome the defect. INH has the structure shown in Fig. 9-11.

Apart from the above, the vitamin is empirically found to be of value in treatment of nausea and vomiting of pregnancy (morning sickness), radiation sickness and muscular dystrophy.

Assay: The general methods for other B-complex vitamins are applicable to this vitamin.

Requirements: 2 mg. a day for an adult are considered adequate.

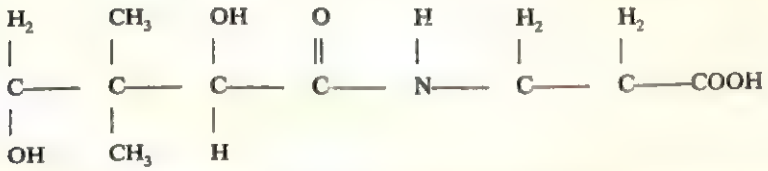
Antivitamins: Deoxypyridoxine (in which the CH_2OH in position 4 is replaced by a $-CH_3$) acts as an antivitamin.

Pantothenic Acid

(Synonyms: Filtrate factor; chick antidermatitis factor).

The meaning of the word 'pantothenic' in Greek is 'from everywhere'. Williams and associates (1933) gave the name to a factor required for the growth of yeast and other microorganisms. Wooley, Weisman, Elvehjem and others showed the identity of the growth factor with the one curing chick dermatitis.

Chemistry: It is α, γ -dihydroxy-β, β-dimethyl-butyryl-β-alanide. It is a pseudopeptide formed by combination of a butyric acid derivative with β-alanine.



It is a viscous, yellow, oily material which is water soluble. Its calcium salt is crystalline and is commonly used in therapy.

Sources: Extensively present in all the common sources of B complex vitamins. It is also present in milk, sweet potatoes and most vegetables. Its richest source is honey.

Metabolism and functions: It is readily absorbed from intestines and excreted mainly in the urine. Its function is as a component of a very important coenzyme called coenzyme A. This coenzyme has the following structure:

β-mercaptoethylamine-pantothenyl-P
+ P-C_3 phosphorylated ribose-adenine

The second component represents a nucleotide structure. The first component, β-mercaptoethylamine ($HS\,CH_2CH_2NH_2$) forms a pseudopeptide linkage with COOH of the pantothenic acid. The active group in the entire complex molecule is the HS-group. Hence for the sake of ease in representing the molecule of the coenzyme-A, it is abbreviated as HS-CoA.

1. It can readily combine with acyl groups and the compound so formed is metabolically active.

   Eg.: Acetic Acid + HS.CoA → Acetyl-CoA.
   Fatty acid + HS.CoA → Fatty acyl-CoA.

   Similarly succinyl-CoA, benzoyl-CoA, and cholyl-CoA formed from succinic acid, benzoic acid and cholic acid are metabolically active compounds.

2. The active acetate in combination with a protein (acyl-carrier protein, ACP) is required for synthesis of fatty acids.

3. Acetyl-CoA is the form in which a two carbon fragment enters the citric acid cycle for oxidation.

4. Adrenal cortical function is much depressed in a deficiency of the vitamin as acetyl-CoA is required for the synthesis of the steroid ring and hence the adrenal cortical hormones.

5. Succinyl-CoA is involved in citric acid cycle.

6. Fatty acyl-CoA formation is the starting point for the oxidation of fatty acids or for their incorporation into triglycerides or phospholipids.

The bond uniting the acyl or acetyl group to the SH of the coenzyme is a high energy bond (HS ~ CoA).

Deficiency: Due to the widespread occurrence of the vitamin in nature, a deficiency condition does not seem to occur in man. Experimental animals show dermatitis, impaired growth and reproduction, alopecia (loss of hair) degenerative changes in the nervous system, gastrointestinal disorders and fatty livers.

Assay: This is as for other B complex vitamins.

Requirements: 5 to 15 mg. per day are supplied by most diets and are found adequate.

Biotin

(Synonyms: Vitamin H; anti-egg-white-injury factor)

Feeding raw egg white to animals was known to produce certain toxic symptoms. Boas confirmed this by her feeding experiments on rats. The symptoms were not produced when the egg white was cooked and fed. Williams and associates demonstrated the presence of an antivitamin in egg white. It is a basic protein, avidin. Kogl and Tonnis (1936) isolated biotin from dried egg yolk. Gyorgy, du Vigneaud and associates elucidated the chemistry and functions of the vitamin.

Chemistry: It is a heterocyclic compound containing sulphur (See Fig. 9-12). THe COOH may combine by a pseudopeptide linkage with the eta (ε) amino group of lysine to form biocytin which appears to be an active functional form of the vitamin. Avidin is a basic protein in egg white and is capable of forming an unabsorbable complex with biotin.

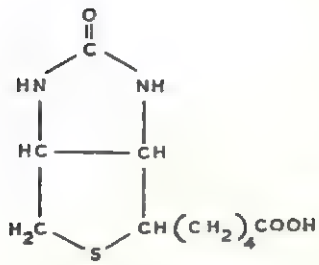


Fig. 9-12. Biotin

Sources: The usual sources of B complex. Honey is a rich source.

Metabolism and functions: Large amounts of the vitamin are synthesized by intestinal flora and are available to the host organism including man.

This important source of supply of the vitamin is lost during therapy with sulfonamides and antibiotics. Avidin of egg white also deprives the availability of the vitamin.

The vitamin is readily absorbed. It is stored in small amounts in liver. Excretion is mainly through urine.

It is a coenzyme involved in CO_2 transfer reactions (or CO_2 fixation).

E.g.: 1. Acetyl-CoA + CO_2 $\xrightarrow[\text{(acetyl-CoA Carboxylase)}]{\text{Biotin}}$ Malonyl-CoA

2. Pyruvic acid + CO_2 $\xrightarrow[\text{(Pyruvic acid carboxylase)}]{\text{Biotin}}$ Oxaloacetate

3. Conversion of beta-methylcrotonyl-CoA to beta-methylglutaconyl-CoA.

$$CH_3C = CH\,COSCoA + CO_2 + ATP \longleftrightarrow HOOCCH_2\,C = CH\,COSCoA + ADP + P$$

4. Similarly, propionyl-CoA is convertible to methylmalonyl CoA and succinyl CoA.

In its function in CO_2 transport, the ureido portion in the upper part of the ring takes up the CO_2 reversibly.

Assay: This is by the usual methods.

Deficiency: Experimentally it can be produced by feeding large amounts of raw egg white. In rat it produces a dermatitis around the eyes giving it a 'spectacle-eyed' appearance. Alopecia, graying of hair, gastrointestinal and nervous symptoms occur in different species. Some of these symptoms occurred in human beings also in experimental deficiency. A deficiency normally does not occur.

Requirements: Unless the intestinal flora are disturbed, the requirements are entirely met by their synthesis. About 100-300 microgrammes may be required daily.

Folic Acid

(*Synonyms:* Liver L-casei factor; vitamin M; Streptococcus lactis R (SLR) factor) vitamin B_c; pteroylglutamic acid; norite eluate factor; vitamin B_{10}; vitamin B_{11}; folacin).

Investigations at University of Wisconsin revealed in 1941 the common nature of a factor required for growth of certain microorganisms and for the chick. This factor was prepared in a concentrated form from spinach and from liver and yeast by different groups of workers. It was obtained in a pure crystalline form and was also synthesized by the investigators in the Lederle Laboratories in 1946. Dr. Yellapragada Subba Rao, an Americanized scientist from Andhra, had the distinction of being associated closely with this work in the Lederle Laboratories.

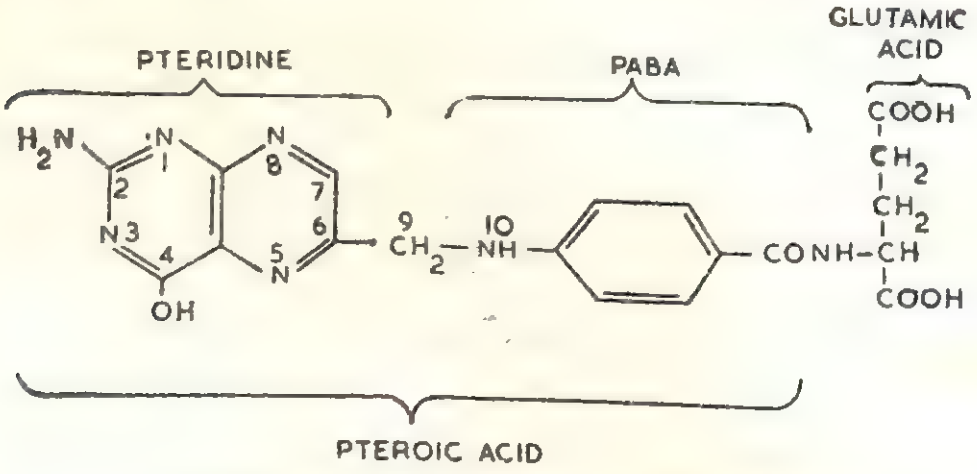


Fig. 9-13.

Chemistry: The term folic acid is derived from its wide spread presence in green leafy vegetables (folium = leaf). Several components having certain characteristic structure exhibit vitamin activity. The common factors in all these compounds are:

(a) pteridine nucleus consisting of pyrimidine and pyrazine rings,

(b) para aminobenzoic acid (PABA) and

(c) glutamic acid. The first two compounds form pteroic acid and all the three together form pteroyl glutamic acid (PGA). More than one glutamic acid may be present per molecule. Compounds with three and seven glutamic acid residues have been isolated. The reduced form of the vitamin (by addition of 4 hydrogens to the pteridine moiety), tetrahydrofolic acid, is the active form of the vitamin. The structures of these different compounds are shown in Fig. 9-13.

Sources: Green leafy vegetables are good sources besides the usual sources of B complex.

Metabolism and functions: Many microorganisms including those inhabiting the intestinal tract can synthesize the vitamin. Some of them cannot synthesize PABA which has to be supplied in the medium and which is hence a vitamin for them. Sulfonamide drugs and antibiotics inhibit their growth by blocking the incorporation of PABA in the folic acid synthesis (competitive inhibition). The higher animals cannot synthesize folic acid and for them folic acid, and not PABA, is the vitamin required. Folic acid is readily absorbed and undergoes reduction in the liver chiefly to tetrahydrofolate, the active form. This is brought about by a folic acid reductase which requires NADPH as coenzyme. Ascorbic acid is also required for the reduction to occur. In the case of the tri and heptaglutamates, a conjugase enzyme will remove all but one glutamic acid residue.

The tetrahydrofolic acid can reversibly combine with a carbon unit at N_5 or N_{10} (in the NH present in these two positions, the H is replaced by one carbon unit) and this seems to be the basis of its function.

Metabolism of One-Carbon Groups: One carbon groups can exist in different states of oxidation.

1. CH_4 (methane): This is inactive in metabolism.
2. CH_3OH (methanol): This can contribute $-CH_3$ groups.
3. HCHO (formaldehyde): This can contribute $-CH_2OH$ (hydroxymethyl) and $-CH_2-$ (methylene) groups.
4. HCOOH (formate): This can contribute –CHO (formyl) and –CH= (methylidine or methenyl) groups.
5. H_2CO_3 (carbonic acid): This can contribute –COOH (carboxyl) or –C=O (carbonyl) groups.

Of these, as already mentioned, CH_4 is inactive metabolically. The role of CH_3OH in contributing CH_3 groups is taken over in mammalian tissues by active methionine (S-adenosyl methionine).

The transfer of hydroxymethyl, formyl, methylene and methylidine groups formed from formaldehyde and formate is mediated by tetrahydrofolate.

The transfer of carbonyl groups from H_2CO_3 is mediated by biotin.

The derivatives are called N_5 or N_{10} methyl (or formyl or hydroxymethyl or formimino) tetrahydrofolic acid. Certain microorganisms of the Leuconostoc citrovorum group can thrive only when the derivative N_5 formyl-tetrahydrofolic acid is present in the medium. Hence this active principle is also called citrovorum factor (CF). Its chemical name is 'folinic acid'.

Leucovorin and rhizopterin are the N_{10} formyl derivatives. The latter does not contain glutamic acid. These compounds can be represented in an abbreviated form thus:

N_5 formyl tetrahydrofolic acid	— $f_5 . FH_4$
N_{10} " " "	— $f_{10} . FH_4$
tetrahydrofolic acid	— FH_4

N_5 and N_{10} forms are interconvertible by the action of an isomerase. Sometimes the formyl group may be shared between the N_5 and N_{10}.

The one carbon moiety can be derived from several sources and can be utilized to form several compounds.

One carbon moiety donors	*One carbon moiety acceptors*
1. Formimino group of formimino glutamic acid (formed from histidine)	1. Positions 2 and 8 of the purine ring
	2. N-formyl methionine of transfer-RNA
2. Methyl group of methionine	3. Glycine to form serine
3. Methyl group of betaine (itself a derivative from choline)	4. Homocysteine to form methionine
4. Methyl group of thymine	5. Uracil to form methionine
5. Beta carbon of serine	6. Ethanolamine to form choline
6. Glycine, tryptophan, amino levulinic acid and acetone	7. Histidine

The Folate Cycle: Tetrahydrofolate takes up a one carbon moiety ($-CH_3$) at N_5 position. The methyl group is transferred to cobalamine to form methyl cobalamine which helps methylation of homocysteine to form methionine. The folate is returned to the folate pool to further take part in one carbon transfer reactions. Otherwise, folate will remain trapped with the methyl group and its further functioning is impaired. This close correlation of folic acid and B_{12} is called the 'Folate Cycle'

The excretion of folic acid is mainly by the kidney. Feces contain large amounts of unabsorbed folic acid synthesized by intestinal flora.

Assay: Mainly microbiological methods employing Lactobacillus casei and Streptococcus fecalis are used.

Deficiency: With normal intestinal flora and in the absence of gastro-intestinal absorption defects, a deficiency is rare. But under conditions of sulfonamide or antibiotic therapy or in case of malabsorption, deficiency symptoms occur. Since the vitamin is very much involved in the synthesis of purine ring which is a component of nucleic acids, and methylation of the pyrimidine ring to form thymine, a constituent of DNA, the most affected function will be

cell multiplication. The hemopoietic system will be the earliest to be involved because it shows the maximum rate of cell division and multiplication normally. Macrocytic anemia, leucopenia and neurological symptoms are produced. Gastrointestinal symptoms also occur.

A metabolite of histidine, formiminoglutamic acid (figlu), which requires folic acid for its further metabolism is excreted in large amounts in conditions of deficiency of the vitamin. Estimation of 'figlu' in urine is hence used to ascertain if a folic acid deficiency exists. Folic acid has been found to be therapeutically of use in the treatment of sprue–a condition of malabsorption.

Daily requirements: 300 to 500 micrograms are adequate to maintain normal health. Intestinal microorganisms supply several times the amount.

The Nutrition Expert Group (ICMR) recommends 100 micrograms per day for an adult.

Folic acid antagonists: Several antagonists to this vitamin were discovered. They are of clinical interest on account of their ability to inhibit cell division and multiplication. They are used in treatment of conditions where there is unrestricted cell growth *eg:* leukemia, erythremias and malignant growths.

Aminopterin (in which the OH group at position 4 of folic acid is substituted by an NH_2 group) is a potent antivitamin. Amethopterin is another. It is 4-amino, 10 methyl folic acid.

Trimethoprim inhibits the enzymic conversion of dihydrofolate to tetrahydrofolate by gram-negative bacteria and thus inhibits bacterial growth.
Methotrexate, on the other hand, inhibits the enzyme in bacteria, as well as mammals.

Vitamin B_{12}

(Synonyms: Cobalamin; cobamide; anti-pernicious anemia factor; extrinsic factor of Castle.)

Rickes and others isolated the anti-pernicious anemia factor from liver extract in 1948.

This was later named vitamin B_{12} after establishing the similarity in its function to that of vitamins. The factor is identical to what were described as 'animal protein factor' and 'animal growth factor'

Chemistry: The structure of cobalamin resembles that of heme in several respects as may be seen from Fig. 9-14.

1. It has four pyrrole rings I, II, III and IV, three of which are linked by methylene bridges, but the I and IV rings are linked directly. The tetrapyrrole ring structure of vitamin B_{12} is called the 'corrin' ring system.
2. Instead of iron, there is trivalent cobalt atom in the centre of the molecule linked by co-ordinate linkages to the nitrogens of the pyrrole rings.
3. The cobalt is also linked to cyanide and the azole ring of a nucleotide (5, 6-dimethyl-benzimidazole 1-α-D-riboside -3 phosphate) which is esterified through amino propanol which in turn is attached to propionic acid residue of ring IV.

'OH' 'NO_2', 'CI' or 'SO_4' may replace 'CN' in which case it is called hydroxy-cobalamin, nitrito-cobalamin, chlorcobalamin, , sulfatocobalamin etc. All of them have vitamin activity, but to a lesser degree than cyanocobalamin. The vitamin is purified to a crystalline substance.

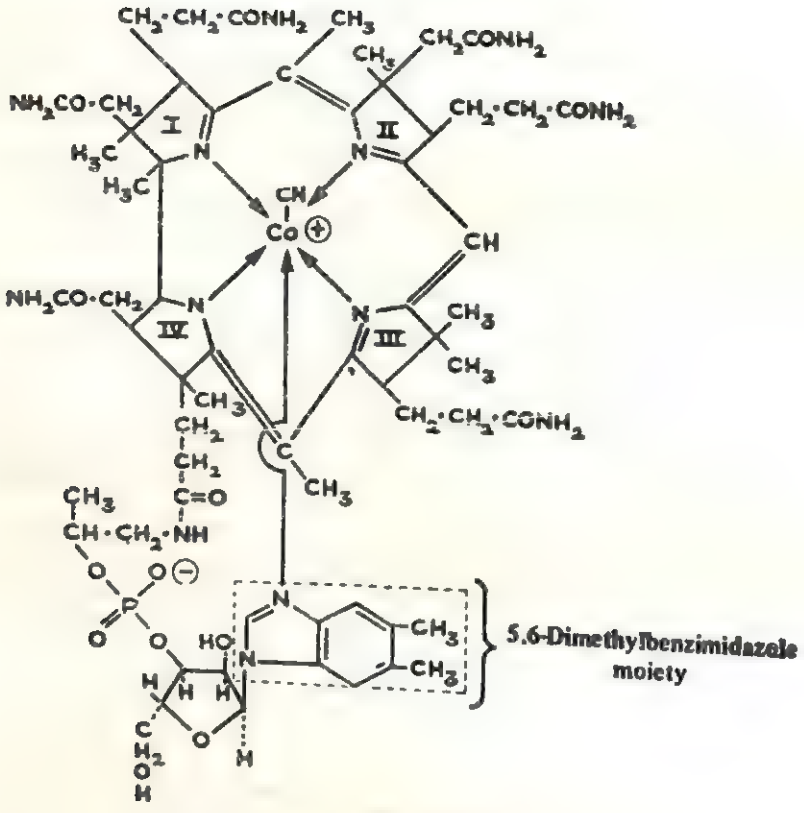


Fig. 9-14. Cyanocobalamin (Vitamin B_{12})

The cobalamins can readily conjugate with proteins, the chief of which the 'intrinsic factor'.

Sources: Liver, eggs, milk, meat and fish contain the vitamin. Liver and kidney are good sources. Little or none occurs in plant materials. Large quantities are present in the rumen of herbivorous animals owing to microbial synthesis. In fact, the primary source of the vitamin is microbial synthesis in the intestine. This is absorbed by higher animals.

Metabolism and functions: The vitamin is absorbed from the intestine only in the presence of the 'intrinsic factor' which is a non-dialyzable, thremolabile substance present in normal gastric juice. It is probably a mucoprotein. The intrinsic factor is said to free the vitamin from protein compiexes and is said to aid its intestinal absorption in co-ordination with a releasing factor present in the intestinal juice. The intrinsic factor varies in chemistry from species to species. It appears to be similar in hog and human. Administration of very large doses of B_{12} may result in absorption of a fraction of it by the intestine even in pernicious anemia (in the absence of intrinsic factor). But normal amounts present in food are not absorbed. Hence the anemia results. Intrinsic factor also favours retention of the vitamin by the tissues. B_{12} derivatives are transported in plasma bound to specific carrier proteins called 'Transcorrin I' and 'Transcorrin II'.

Excretion: Mainly through the bile. There is some degree of enterohepatic circulation. Feces contains the unabsorbed vitamin and the vitamin excreted through bile. In addition, it may contain large amounts synthesized by the colonic bacteria.

Urinary excretion is slight due to protein-binding of most of the circulating vitamin. However, following parenteral administration, there is urinary excretion.

The cobalamins function as coenzymes in several reactions:

The vitamin functions as coenzyme after combining with an adenosyl moiety which replaces the –CN group. The pentose of the adenosine moiety is deoxyribose, and the coenzyme is called 'cobamide coenzyme'.

1. Conversion of methyl malonyl-CoA to succinyl-CoA (in propionic acid metabolism in animal tissues).
2. Interconversion of glutamate and β-methylaspartate (in bacteria).
3. Methylation of homocysteine to methionine (requiring tetrahydrofolic acid as the methyl carrier). The vitamin may be actively involved in conversion of one carbon units eg: CHO to CH_2OH or CH_3 and the methylation of pyrimidine ring to form thymine.
4. It is also necessary in the metabolism of diols *eg:* ethylene glycol

$$\begin{array}{l} CH_2OH \\ | \\ CH_2OH \end{array} \rightarrow CH_3CHO + H_2O$$

5. Conversion of ribonucleotides to deoxyribonucleotides. This function is of importance in synthesis of DNA in some bacteria.

Like folic acid, B_{12} is also intimately connected with hemopoiesis. Urine of B_{12} deficient humans contains large amounts of methyl malonic acid.

Deficiency: The most important deficiency symptom is macrocytic anemia. This is associated with lesions of the CNS described as subacute combined degeneration. The exact mechanism is not known, but it may be on account of the interference with the normal synthesis of nucleic acids, particularly DNA.

A deficiency due to dietary lack is uncommon on account of its wide-spread occurrence in all animal foods and synthesis by intestinal flora. A deficiency of the 'intrinsic factor' is more likely to be the cause of B_{12} deficiency. The condition is called pernicious anemia. Intrinsic factor deficiency is usually associated with absence of HCL (achlorhydria) and enzymes (achylia) in gastric juice.

Confirmatory test for pernicious anemia:

If 0.5 microgram of cyanocobalamin labelled with ^{60}Co, is administered orally, only 5-30% of the radioactivity is recovered in the stool in a normal subject. In pernicious anemia, 75-90% of the dose is recovered in the stool.

Assay: Microbiological using Lactobacillus leichmannii, E-coli and others. Spectrophotometric methods are also available.

Requirements: National Research Council, U.S.A., recommended a daily intake of 5 micrograms. But, the Nutrition Expert Group (ICMR), taking into account the actual B_{12} consumption of the Indian subjects, recommended I microgram per day as adequate.

Vitamin 'C' or Ascorbic Acid

Scurvy was known for centuries. Lind gave accurate description of the disease as early as 1757. English sailors knew that the disease could be prevented by taking fresh lime juice. Szent-Gyorgi in 1928 isolated a substance from adrenal gland called hexuronic acid which was later identified as vitamin C by Waugh and King (1932).

Chemistry: It is a white crystalline substance with a very acidic taste. It has a structural resemblance to hexoses. It is a strong reducing substance on account of its enediol structure. It is stable in the solid form and in acidic solutions, but is rapidly destroyed in alkaline solutions. The presence of traces of copper will accelerate the process further.

While the conversion to dehydroascorbic acid is a reversible change, the further hydrolysis of this compound to *l*-diketogulonic acid is irreversible (see Fig. 9-15).

Sources: Fresh green vegetables and salad vegetables like cabbage, lettuce, spinach, amaranth and cucumber contain the vitamin. The citrus fruits (lemons and oranges) and also berries and melons are particularly rich in the vitamin. Tomatoes and potatoes also contain good amounts. Gooseberry is one of the richest sources of this vitamin.

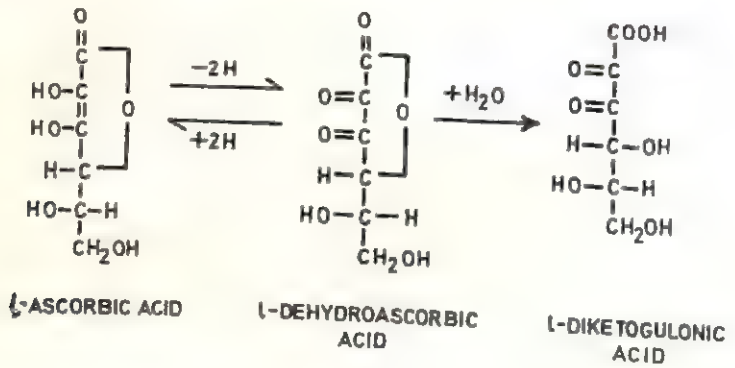


Fig. 9-15, Ascorbic Acid and Derivatives

Metabolism: The vitamin is rapidly absorbed from gastrointestinal tract. There is very little storage. It is excreted in the urine partly unchanged and partly as the diketogulonic acid and further breakdown products like oxalic acid. In the body, the ascorbic acid and small amounts of the dehydro form exist in equilibrium with one another.

Biosynthesis of the vitamin: Some of the lower animals (e.g. rat) can synthesize the vitamin from glucose by the uronic acid pathway. The synthesis involves an inversion whereby the C_1 and C_6 of D-glucose become the C_6 and C_1 of *l*-ascorbic acid. Man, monkey and guinea-pig lack the full complement of the enzymes required for the synthesis and hence have to be supplied the vitamin in diet.

Functions: The vitamin is intimately concerned in the metabolism of the mesenchymal tissues, osteoid, dentine and collagen.

1. The readily reversible conversion to the oxidized form of dehydroascorbic acid probably enables it to function in maintaining the redox potentials of the cell. It probably plays the role of a coenzyme in hydrogen transfer reactions involving NADP and glutathione.

2. *Collagen Synthesis:* Hydroxyproline is an important amino acid in collagen. The formation of hydroxyproline by hydroxylation of proline requires vitamin C. It is probably required for other hydroxylation reactions as well eg: Tryptamine → 5-hydroxytryptamine or serotonin.

3. *Tyrosine metabolism:* Guinea pigs deficient in ascorbic acid excrete P-hydroxy phenylacetic acid in urine. The conversion of the substance to homogentsic acid requires ascorbic acid.

4. Ascorbic acid in food helps in the absorption of iron by converting the inorganic ferric iron to the ferrous form.

5. Adrenal cortex shows high concentrations of the vitamin. Stimulation of the gland by ACTH will cause a rapid depletion of the vitamin indicating that it is required for the normal function of the gland.

Deficiency: In the human, its deficiency produces a disease called 'scurvy' The main defect is a failure to deposit intercellular cement substance. The capillaries are fragile and there is a tendency to hemorrhage. Wound healing is delayed due to deficiency in the formation of collagen. There is poor dentine formation in children leading to poor teeth formation. Gums are swollen and spongy and bleed on slightest pressure. Osteoid of bone is poorly laid and hence mineralization also is poor. The bones are weak and readily fracture. Hemorrhages occurring below the periosteum and into the joints cause extremely painful swellings of bones and joints.

There is also a mild degree of anemia.

Requirements: 30 mg. for infants and 70 mg. for adults are recommended by the National Research Council. More is required during pregnancy and lactation. The Nutrition Expert Group (ICMR) has recommended 50 mg. per day as adequate for the Indian adult.

Assay: A chemical method by titration with a dye, 2, 6-dichlorophenol-indophenol, is widely used. Colorimetric estimation of the complex formed with 2, 4-dinitrophenyl-hydrazine is another method.

The vitamin C status of an individual (whether a person is having an adequate dietary supply or is starved of the vitamin) can be tested by administering a test dose of 5 mg. per lb. body weight. If 50% or more is excreted in the next 24 hours, he has no deficiency of the vitamin.

Tourniquet test is another where a rubber band is tied around the arm to compress the venous flow. In a short time several petechial hemorrhages appear on the forearm visible under the skin.

TABLE 9–1
Vitamins A and D content of some foods

	Vitamin A I.U./100 gm.	*Vitamin D I.U./100 gm.*
Milk	110	1
Butter	4,000	60
Ghee (cow)	2,000	
Ghee (buffalo)	1,000	
Egg	1,000	60
Heart	200	nil
Kidney	1,000	nil
Liver	15,000	nil
Fish	150–250	500–1,000
Cod-liver oil	100,000	20,000
Halibut-liver oil	5,000,000	200,000
Shark-liver oil	12,000,000	500-2,500

Note: Carotenes, the precursors of vitamin A, are present in many green vegetables and some fruits and seeds. The carotene content along with thiamine, riboflavin, niacin and vitamin C are listed in Table 27-4. the requirements of the more important vitamins under different conditions are listed in Table 27-5.

References:

Baker, H.: Clinical Vitaminology., Interscience., N.Y., 1968.
Davidson, S., Meikle John, A.P. and Passmore, R: Human Nutrition and Dietetics., Livingstone, Edin., 1959.
Dyke, S.F.: The Chemistry of the Vitamins., Interscience., N.Y., 1965.
Gopalan, C. and Narasinga Roa, B.S.: Dietary Allowances for Indians., I.C.M.R. Spl. report series No. 60, Hyderabad, 1971.
Gopalan, C., Rama Sastri, B.V. and Balasubramaniyan, S.C.: Nutrition Values of Indian Foods., Natl. Inst. Nutr., I.C.M.R., Hyderabad, 1971.
Gyorgy, P. and Pearson, W.N.: (eds): The Vitamins, vols. 6 & 7., Acad. Pr., N.Y., 1967.
Heinz Handbook of Nutrition, McGraw-Hill, N.Y., 1965.
Robinson, F.A.: The Vitamin Cofactors of Enzyme Systems, Pergamon Pr., N.Y., 1966.
Sebrall, W.H. Jr. and Harris, R.S. (eds.): The Vitamins., 2nd. ed., Vol. 1-5, Acad. Pr. N.Y., 1967.
Wagner, A.F. and Folkers, K.: Vitamins and Coenzymes, Interscience, N.Y., 1964.
Williams, R.J. et al: The Biochemistry of the B-Vitamins., Reinhold, N.Y., 1950.
Recommended Dietary Allowances., Publication, 1964., Natl, Acad. Sc. Natl. Res. Council (U.S.A.) 1968)

10

DIGESTIVE SECRETIONS, DIGESTION AND ABSORPTION

THE principal constituents of food, namely carbohydrates, lipids and proteins are consumed by the higher animals including man, in a form essentially different from that obtaining in their tissues. They undergo suitable alterations whereby they are broken down into simple units which can readily be absorbed by the intestines and can be used by the tissues for resynthesis into their characteristic tissue lipids, proteins etc. These processes are known as digestion and absorption. The secretions of the salivary glands and the gastrointestinal tract contain enzymes suitable for digestion of the various components of food. Cooking of food is necessary in many cases to prepare the food for enzyme action. It will break the cellulose walls of vegetable matter, convert the insoluble starch to a soluble form and insoluble collagen to soluble gelatin, and also bring about increased palatability and taste to the food materials. Physical factors like mastication (chewing), movements of the stomach and intestine help in grinding the food material to small particles and bringing them into intimate contact with the digestive juices. Nature has provided an array of enzymes to act in succession on the food materials so that all digestible material is completely digested during its passage through the gastrointestinal tract. It has also provided a length of small intestine specially adapted for the absorptive process. It is about 27 feet long in the human and the mucus membrane is thrown into several folds and is studded with over five millions of fingerlike processes called villi, thus providing about 10 sq. meters of surface for absorption of the digested products. This enables almost complete absorption of the products of digestion by the intestines during the five to eight hours required for the passage of the material into the large bowel. The villus is the primary structure concerned with **absorption**. It has a brush border for greater ease of absorption. Leading from it are lymphatics which are provided with valves and lead into the thoracic duct which in turn empties into the subclavian vein and thus into systemic circulation. There is also a capillary network which empties into a radicle of the portal vein which reaches the liver.

The gastrointestinal tract is open at both ends and can be considered to be in continuity with the external environment, but so compartmentalized and suitably walled to make it possible for processing the material before it enters the system (blood and tissue). It has an analogy to the small invaginations produced in the cell wall engulfing materials from the surrounding medium to form a vacuole or cell-inclusion into which enzymes are poured in. The gastrointestinal tract is a permanent invagination which can be sealed off though imperfectly, at the oral and anal ends.

To facilitate easy comprehension, the chemical composition of the different digestive secretions will be first dealt with. This will be followed by a discussion of the digestion and absorption of each of the principal constituents of food.

SALIVA

It is a mixture of secretions by three pairs of salivary glands–the parotid, the submaxillary and the sublingual. It is a colorless, opalescent, slimy fluid. About 1,000–1,500 ml are secreted per day. The stimuli for secretion are psychic (eg: sight, smell or thought of food) or mechanical (chewing) or chemical (due to stimulation of taste buds).

It contains 99.4% water and 0.6% solids and is secreted at a pH of about 6.8. About a third of the solids are inorganic (chloride and bicarbonate of sodium mainly; smaller amounts of potassium and calcium salts; traces of iodide and thiocyanate). Two thirds of the solids are organic and consist mainly of the enzyme salivary amylase (or ptyalin) and mucin. Mucin is a glycoprotein and it helps in lubricating the food in its passage down by giving it a slimy consistency. It is mainly produced by the sublingual and submaxillary glands. The enzyme is mainly from parotids. Other organic substances like urea, glucose and lactic acid are present in small amounts.

The chief function of saliva is as a lubricant. The lubrication is required not only for swallowing food but also for movements of tongue as in speech. It also helps in dissolving the food materials and enabling them to stimulate the taste buds and create the various sensations of taste. Amylase exerts a digestive action on carbohydrates.

GASTRIC JUICE

This is a mixture of the secretions of the parietal cells, the chief cells and columnar cells. The former two are present in the tubular portion of the gastric glands and secrete HCL and enzymes mainly. The columnar cells are present near the neck of the gastric glands and add mucus to the secretion. The combined secretion is called the gastric juice. 2,000–3,000 ml. are secreted per day. Uncontaminated gastric juice is a clear, colorless liquid containing 99.4% water and 0.6% solids. The chief inorganic constituent is HCL. Small amounts of NaCL, KCL and phosphate are present. Mucin and the proteolytic enzyme pepsin are the main organic constituents. The pepsin is secreted as the inactive form pepsinogen by the chief cell but is converted to pepsin in the presence of HCL of the parietal cell secretion. It is a proteolytic enzyme and is considered further under digestion. Small amounts of lipase and rennin are the other enzymes present. But these are of significance only in the newborn infant where the gastric pepsin and HCL are low. The lipase is of no utility in the highly acid medium of the adult gastric juice. The milk curdling property of rennin is not required in the adult since pepsin has got a similar effect. In the newborn infant, however, curdling of milk is necessary to keep it long enough in the stomach for digestion and to prevent rapid emptying of the stomach.

HCL: The secretion, as comes out from parietal cells, is a solution of pure HCL of a concentration of about 170 m. eq/litre (0.17N) with a pH of about 0.87. This undergoes dilution to a varying extent depending on the magnitude of the secretion of chief cells and mucus secreting columnar cells. The mucin of the latter also exerts a buffering action on the

acid. The hydrochloric acid functions by:

1. providing the optimal pH for pepsin action,
2. activating pepsinogen to pepsin,
3. causing denaturation of proteins of food which renders them more easily digestible,
4. facilitating absorption of iron by converting colloidal iron into ionic form,
5. stimulating duodenum to liberate secretin and
6. acting as germicide.

Gastric mucus: In addition to mucin secreted by the columnar epithelium at the neck of gastric glands, the epithelial lining of the rest of the gastric mucosa also secretes mucinous material all of which is collectively called gastric mucus. Gastirc mucus is resistant to peptic digestion and exerts a protective action on the mucus membrane. The intrinsic factor of Castle is associated with gastric mucus.

Stimulation of gastric secretion: This is said to occur in three phases:

(i) Cephalic phase: The taste, smell or sight of food will reflexly invoke sercetion of gastric juice.

(ii) Gastric phase: The presence of food in the stomach will stimulate its secretion. The stimulus is mainly chemical and will cause liberation of a hormone called 'gastrin' by the pyloric mucosa which is absorbed into the blood and reaches the gastric glands and stimulates them. Histamine has similar stimulating action on gastric glands when injected parenterally and gastrin was considered to be identical with histamine. But recently gastrin was isolated in a pure state by Gregory and Tracy (1961) and was shown to be different from histamine. Two different substances gastrin I and gastrin II were isolated by them from hog antral mucosa. They are both polypeptides.

(iii) Intestinal phase: When certain foods are placed directly in the duodenum, gastric secretion occurs. This also is said to be due to hormonal stimulation.

Inhibition of gastric secretion: If fat is introduced into stomach, it inhibits gastric secretion. The effect is even more if fat is introduced into duodenum directly. It is said to be also a hormonal action by a substance 'enterogastrone' liberated by the duodenal mucosa.
The secretion of gastric juice including HCL is inhibited. Gastric motility is also diminished. Urine contains a similar inhibitory substance called 'urogastrone'

Secretion of Gastric HCL

Gastric juice is exceptional in that it is the only secretion which is highly acidic. HCL is secreted by the parietal cell at a concentration of about 170 m.eq./litre and is isotonic with blood plasma. It is a pure solution of isotonic HCL.

The Cl^- from plasma is pumped by an active transport process into the lumen by the parietal cell. The lumenal surface of the cell therefore becomes electronegative (due to Cl^-), with respect to the serosal surface. A K^+ -ATPase located in the plasma menbrane will pump H^+ ions into the lumen in exchange for K^+ ions. The breakdown of ATP provides the necessary energy. This pump is stimulated by K^+ and Mg^{++}, but not by Na^+. Hence, it is different from the usual Na^+, K^+ -ATPase. The H^+ that is secreted is replaced by H^+ supplied by the carbonic anhydrase action.

$$H_2O + CO_2 \longrightarrow H_2CO_3 \longrightarrow H^+ + -HCO_3$$

The HCO_3^- is absorbed into the plasma to replace Cl^-.

Secretion of HCL by the parietal cells is regulated by nervous and hormonal mechanisms. Vagal stimulation increases secretion of gastric HCL. A group of hormones –*gastrins*– produced by the pyloric antral mucosa, stimulate the secretion of HCL. Secretin and somatostatin inhibit the secretion of gastrin. Several peptide hormones -eg. gastric inhibitory peptide, cholecystokinin, somatostatin, vasoactive intestinal peptide and urogastrone - all inhibit gastric acid secretion.

Histamine stimulates acid secretion. It binds to a specific receptor and stimulates adenylate cyclase. The cyclic AMP formed as a result will stimulate a low activity isoenzyme of carbonic anhydrase and causes secretion of acid. *Cimetidine* competitively inhibits the binding of histamine to receptors. It also attenuates the response to gastrin and vagus stimulation, thereby effectively bringing down gastric acidity.

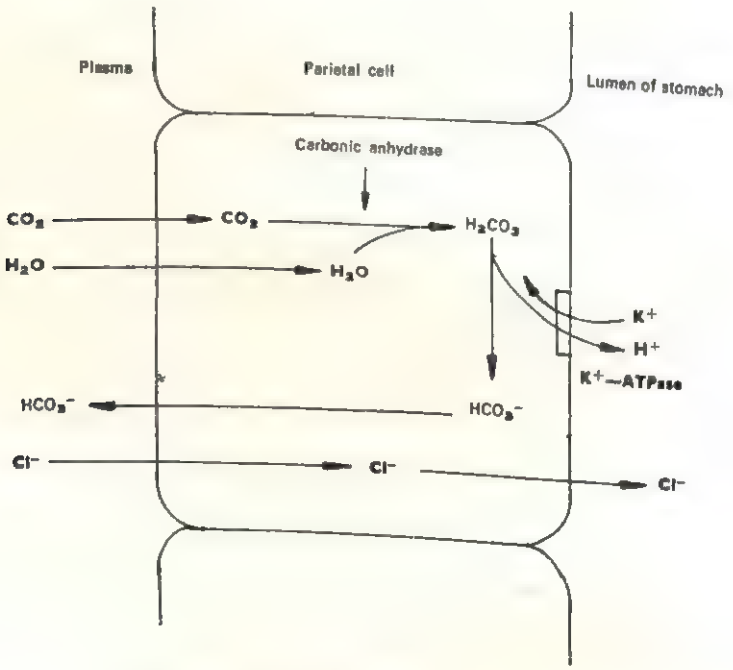


Fig. 10-1. Secretion of HCL by Parietal Cell

Gastric Analysis: A Ryle's tube is passed into the stomach in the morning (on an empty stomach) and the gastric secretion is continuously aspirated. The basal output of HCL is measured. An injection of histamine or pentagastrin (a synthetic analogue of gastrin) is injected subcutaneously and the gastric juice is collected over a further period. In a normal individual –

basal output is 1-5 m.eq./litre and maximal output after stimulation is 10-40 m.eq./litre. In patients with duodenal ulcer, basal output is 2-16 m. eq./litre and maximal output 15-60 m.eq./litre. In Zollinger-Ellison syndrome, basal output may be as high as 10-30 m.eq. litre.This is due to ectopic production of gastrin by pancreatic islet cell tumours.

Alkaline tide during gastric secretion: Owing to secretion of a large amount of H^+ as HCL, there is surplus of OH^- in the parietal cell which is taken up not only by the CO_2 to form HCO_3^- but also by other buffer systems of the parietal cell initially and later by those of plasma.

$\frac{HPO_4^{=}}{H_2PO_4^-}$ $\frac{HCO_3^-}{H_2CO_3}$ $\frac{\text{Lactate}}{\text{Lactic acid}}$ all tend to increase on the side of the

base ($HPO_4^{=}$, HCO_3^- and lactate$^-$) with the result that the pHof plasma is raised and an alkaline urine is excreted for some hours following intake of food and gastric secretion. This is known as the alkaline tide.

PANCREATIC JUICE

This is also a clear watery secretion. It contains 1.5% solids and 98.5% water. It has a pH of 7.0 to 8.0. About 500–800 ml are secreted per day. Two thirds of the solids are organic and consist mainly of the enzymes trypsinogen and chymotrypsinogen which are zymogen (inactive) forms of the proteolytic enzymes trypsin and chymotrypsin. An enzyme produced by the intestines called 'enterokinase' acts on these inactive enzymes and converts them to the active zymase forms. The activation involves hydrolytic removal of a small peptide from the inactive enzymes. Once active trypsin is produced thus in small amounts, it can activate the rest of trypsinogen and chymotrysinogen (autocatalysis). Another proteolytic enzyme called carboxypeptidase (secreted probably as a procarboxypeptidase and activated by trypsin) is also present. A lipolytic enzyme – pancreatic lipase or steapsin – is another very important enzyme of pancreas. There is also a potent pancreatic amylase. Besides these principal enzymes, there are also other enzymes – cholesterol esterase, ribonuclease, deoxyribonuclease, collagenase and phospholipases.

One third of the solids (0.5%) are inorganic and consist mainly of the chloride and bicarbonate of sodium. Small amounts of potassium, calcium and phosphate are also present.

Stimulation of pancreatic secretion: The entry of partially digested semisolid food (chyme) from stomach into duodenum stimulates secretion of 'secretin' by the duodenum. Secretin is a hormone and is carried by blood to the pancreas where it stimulates its secretion. The secretin itself is said to exist as prosecretin in the duodenal mucosa. The acid (HCL) present in chyme converts this to secretin. It is a small polypeptide containing 27 amino acids and has a low molecular weight (5,000). Another hormone called pancreozymin is also produced by intestines. While secretin stimulation produces a bicarbonate-rich pancreatic juice, pancreozymin induces an enzyme-rich secretion. Stimulation of vagus also induces an enzyme-rich secretion. Pancreatic juice can be collected and studied by introducing a duodenal tube, removing the resting contents and then injecting secretin to stimulate pancreatic secretion.

INTESTINAL JUICE OR SUCCUS ENTERICUS

It is a mixture of secretions by the different intestinal glands (glands of Brunner and Lieberkuhn) present in the small intestinal mucosa of duodenum, jejunum and illeum. It also contains desquamated epithelial cells, leukocytes and mucus. The solids constitute 1.5% and 98.5% is water. Half the solids are organic – mucin, cholesterol, phospholipids, lipids and enzymes – namely nucleotidase, nucleosidase, maltase, lactase, sucrase, aminopeptidase, dipeptidase, phosholipases and phosphatases. Some are secreted by the intestinal glands. Others are present in the intestinal epithelial cells (brush border) and act locally on the substances reaching them.

The inorganic substances are similar to those of pancreatic juice. The pH also is similar i.e., 7.0 to 8.0. The quantity secreted per day may amount to 2,000–3,000 ml.

Like pancreatic and gastric juices, the intestinal juice also is secreted on stimulation by enterocrinin, an intestinal hormone produced by duodenum and jejunum.

The mucus membrane lining the gastrointestinal tract is protected from auto digestion by the following mechanisms:

1. All enzymes are stored in the mucosal cells and secreted in an inactive, zymogen form. Activation occurs only in the lumen of the gastrointestinal tract.
2. The mucus membrane is protected by a layer of mucus which resists enzyme permeation and digestion.
3. The brush border of the intestinal mucosa is covered by a membrane which is lined on the lumenal side by a carbohydrate-rich layer called the *glycocalyx*, rich in neutral and amino sugars. This is highly resistant to digestion by the gastrointestinal enzymes.

THE BILE

Strictly this is not a digestive secretion since it does not contain any digestive enzyme. But it is secreted into the duodenum at the same site as pancreatic juice and it aids in digestion and absorption of lipids in particular. It is secreted continuously by the liver and is collected into the

TABLE 10-1

Constituents	*Gall bladder bile*	*Hepatic bile*
Water ...	88.9%	97.3%
Solids ...	11.1%	2.7%
Bile acids ...	5.2%	1.1%
Mucin and bile pigments ...	3.4%	0.6%
Total lipid ...	2.3%	0.3%
Neutral fat ...	0.4%	0.1%
Fatty acids ...	1.0%	0.1%
Phospholipids ...	0.2%	below 0.1%
Cholesterol ...	0.6%	0.1%
Inorganic substances ...	0.9%	0.8%
pH ...	5.5 to 7.0	7.0 to 8.5
Specific gravity ...	1.040	1.010

gall bladder, where it is stored and undergoes certain changes by way of reabsorption of some substances. Large amounts of water are reabsorbed leading to a concentration of bile in the gall bladder. The water is absorbed along with inorganic components as isotonic solution. Hence, the organic constituents like cholesterol, mucin and bile pigments get concentrated in gall bladder bile while inorganic substances are practically same. About 500–1,000 ml of bile are secreted by liver in a day. The composition of the bile as secreted by liver and as modified by storage in the gall bladder is tabulated in Table 10-1.

Of these different constituents, bile acids are the most important from the point of view of digestion. They are cholanic acids carrying one or more hydroxy groups. The cholanic acid has a steroid structure, but the side chain is shortened and ends in a carboxylic group (see Fig. 10-2).

3,7,12 trihydroxy cholanic acid is cholic acid.

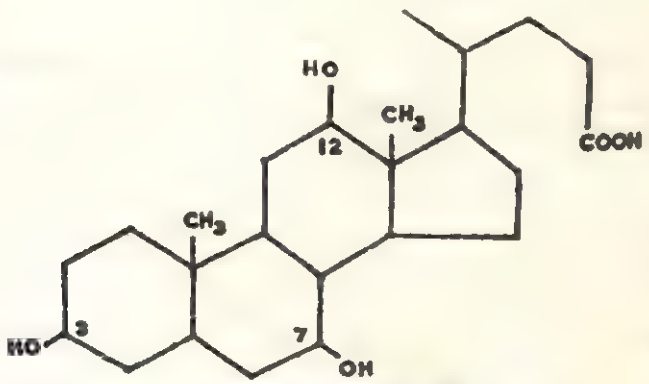
3,7 dihydroxy cholanic acid is chenodeoxy cholic acid.

3,12 dihydroxy cholanic acid is deoxycholic acid.

3 hydroxy cholanic acid is litho-cholic acid.

They are conjugated with either glycine (NH_2 CH_2 COOH) or taurine (NH_2 CH_2 CH_2 SO_2 OH, a derivative of cysteine). The linkage is between the NH_2 group of these and the -COOH of cholic acid by what is known as a pseudopeptide linkage.

The bile acids are produced in the liver itself by oxidation of cholesterol. They are secreted as the potassium or sodium salts known as bile salts. They aid in the digestion and absorption of fat and fat soluble vitamins and are partly reabsorbed with them to be resecreted again. This is known as enterohepatic circulation of bile salts.



3, 7, 12 — TRIHYDROXY CHOLANIC ACID (CHOLIC ACID)

Fig. 10-2

Liver synthesizes about 200–500 mg of bile acids per day to replace the daily loss of these substances in the feces. If the enterohepatic circulation of bile acids is prevented by administering a resin 'cholestyramine' which binds the bile acids and prevents their absorption, there is

increased excretion of bile acids in the feces. More of cholesterol is then oxidized to form bile acids to replace the larger fecal excretion. This is one method to bring down plasma cholesterol levels.

Functions of bile salts

1. On account of their ability to lower surface tension of water, they act as powerful emulsifying agents on the fat present in food. The emulsification is necessary for the action of lipase on fat.
2. In the presence of bile salts, a colipase (M.W. 10,000) binds to lipase and shifts the optimal pH of the enzyme from 9.0 to 6.0, which is the pH usually attained in the small intestine.
3. They form complex (miscelles) with fatty acids, mono, di-and triglycerides which are water soluble and absorbable.
4. They stimulate intestinal peristalisis.
5. They also stimulate bile production by liver (cholagogue effect).

Gall stones: The gall bladder being a *cul de sac* connected to the main flow of bile in the common hepatic duct and on account of the high degree of concentration to which the bile constituents are subjected to, sometimes they get precipitated out to form what are known as gall stones. Infection and other conditions favour their formation. Stones of several varieties occur – cholesterol stones, pigment stones, calcareous stones and so on.

Cholecystokinin is a hormone produced by duodenum and upper jejunum. It causes the gall bladder to contract and empty its contents into the intestines. Cholecystokinin is now found to be no other than pancreozymin.

Bile pigments: Biliverdin and bilirubin are the principal bile pigments. They are excretory products of heme metabolism. The human erythrocyte has a life span of about 120 days, at the end of which it is broken down in the reticuloendothelial system – the bone marrow, spleen and liver (Kupfer cells). The hemoglobin liberated from the erythrocyte is hydrolyzed in the reticuloendothelial cells to form the protein globin and the iron containing porphyrin 'heme'. The tetrapyrrole complex ring structure is opened out by breaking the alfa methylene bridge. This structure, which still contains iron, is called verdohemin. The iron is next removed giving rise to biliverdin. In this, the four pyrrole rings are united by three methylene bridges (the original beta, gamma and delta) in a chain form as shown in Fig. 10-5. The iron and the globin are reutilized in the body but the biliverdin is further converted to bilirubin by reduction either in the reticuloendothelial system itself or in the liver. During its passage through blood, the insoluble bile pigments are carried in loose combination with plasma albumin. One gram of hemoglobin yields about 35 mg of bilirubin.

Occasionally the opening of the porphyrin ring (tetrapyrrole) may occur while globin is still with it. It is then called verdoglobin or choleglobin.

Uptake of bilirubin by the liver:

Bilirubin is transported in plasma by albumin. Albumin has two binding sites for bilirubin-one with a high affinity and the other with a low affinity. Upto 25 mg/100 ml plasma can be bound at the high affinity site at normal concentration of plasma proteins. Antibodies and certain drugs compete for the same site on albumin and their administration may release bound bilirubin.

Bilirubin from plasam is taken up by the liver cell at the sinusoidal surface and the uptake is mediated by a carrier molecule. The capacity of the liver cell to take up bilirubin is quite high and is adequate to remove the plasma bilirubin completely even at high plasma bilirubin levels.

Conjugation of bilirubin: This occurs in the smooth endoplasmic reticulum of the liver cell which contains the enzyme 'UDP-glucuronyltransferase' which is capable of adding glucuronate to bilirubin by transfer from UDP-glucuronate. Separate enzymes exist for the addition of the first and the second glucuronate molecules.

Kidney and intestinal mucosa also posses UDP-glucuronyl transferase activity and can conjugate bilirubin.

Secretion of bilirubin into bile: The conjugated bilirubin, mostly the diglucuronide, is secreted into bile against a concentration gradient by active transport. While the ability to take up bilirubin from circulation and to conjugate it are more than adequate even at high levels of plasma bilirubin, the ability to secrete the pigment into bile by the liver cell is limited and is therefore a rate-limting step.

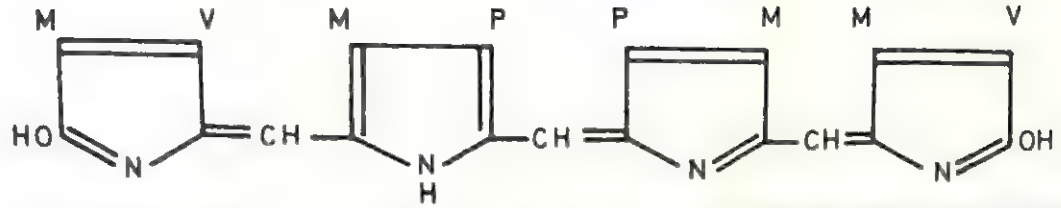
Unconjugated hyperbilirubinemia: In the newborn infant, the UDP-glucuronyl transferase activity is not fully developed. There is also excessive production of bilirubin due to increased hemolysis. Hence unconjugated bilirubin accumulates in blood. When it reaches over 25 mg/100 ml, it exceeds the capacity of albumin to bind it at the high affinity site. It gets released and penetrates the blood-brain barrier of the infant and causes kernicterus and encephalopathy. Administration of phenobarbital is found to induce UDP-glucuronyltransferase activity and is used in treating the condition.

Exposure to visible light also, by some unknown mechanism, promotes excretion of unconjugated bilirubin by liver by converting bilirubin to more readily excretable derivatives.

Crigler-Najjar Syndrome: In this condition, there is a primary metabolic defect in the conjugation of bilirubin, inherited as an autosomal recessive. UDP-glucuronyltransferase activity is absent in the liver cell. In type I of the syndrome, serum bilirubin levels exceed 20 mg/100 ml and the condition is usually fatal in the first 15 months of life. In type II condition, the jaundice is less severe and bilirubin monoglucuronide is present in the bile. The enzyme defect seems to involve only the addition of the second glucuronate. Type II condition shows improvement with phenobarbital.

Gilbert's Disease: This is the name given to a group of diseases where there is excessive hemolysis and unconjugated hyperbilirubinemia. There is some lowering of UDP-glucuronyl transferase activity. But the main defect is in the uptake of bilirubin by the liver cell. The disease is transmitted as an autosomal dominant.

Conjugated hyperbilirubinemia: Chronic Idiopathic Jaundice (Dubin-Johnson Syndrome): The urine contains bile pigments. It is inherited as an autosomal recessive. The hyperbilirubinemia may occur in childhood or in adult life and is due to a defect in the liver cell in the secretion of conjugated bilirubin into bile. The excretion of conjugated hormones



BILIRUBIN

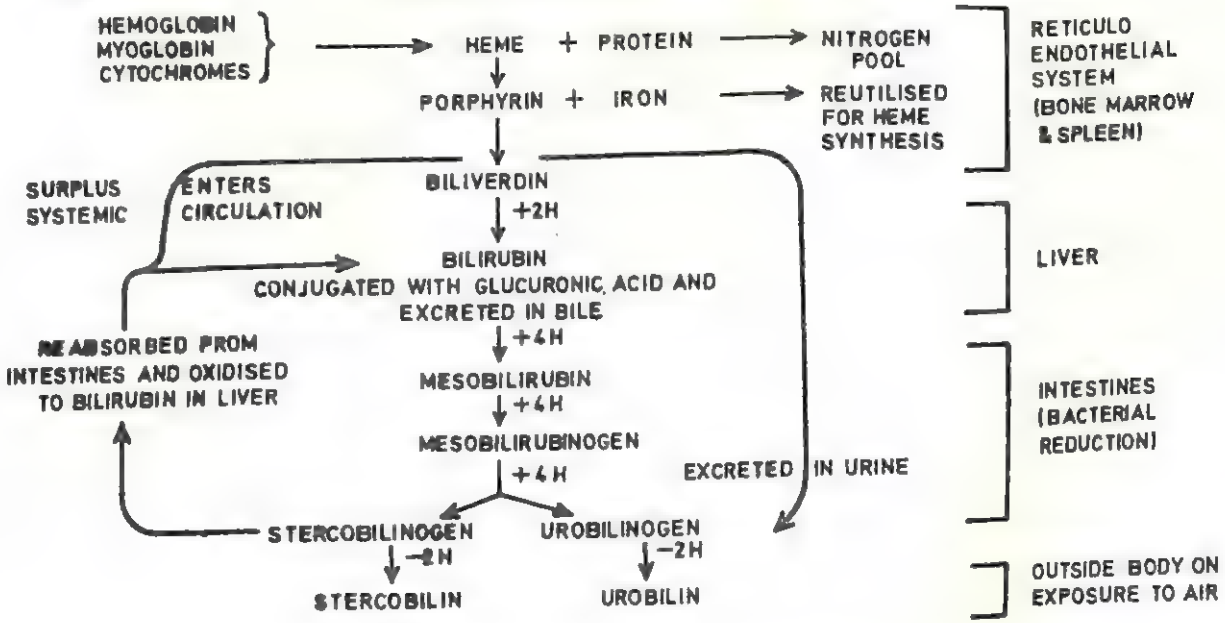


Fig. 10-3 Formation and Excretion of Bile Pigments

(like estrogens) and dyes (like sulfobromopthalein) is also impaired. Dyes which do not require conjugation before excretion (eg. Rose Bengal) are excreted without any difficulty. The excretory pattern of porphyrins in urine is also abnormal in these people. 80–90% of coproporpyrins excreted are of Type I instead of the normal type III.

The bile pigments, on reaching the intestines, are acted upon by reducing bacteria which will reduce bilirubin to mesobilirubin, mesobilirubinogen and then to urobilinogen (or stercobilinogen). Some of this is excreted in feces and is called stercobilinogen. On standing, it is oxidized to stercobilin which is dark colored. This is the reason why feces turns dark some time after passing.

A portion of the urobilinogen is reabsorbed by the intestinal mucosa and reaches back the liver where most of it is reconverted (by oxidation) to bilirubin and reexcreted. A small amount will also escape from the liver into systemic circulation and will be excreted by the kidney as urobilinogen which, on standing, is converted to urobilin by oxidation. A small fraction of bile pigments is also derived from the breakdown of myoglobin and cytochromes which also contain heme. The events are summarized in Fig. 10-3.

A test called Van den Bergh reaction is employed to detect and estimate the conjugated and free bilirubin. Details of the test and the different types of jaundice are discussed in the chapter on 'Assessment of Liver Function'.

DIGESTION AND ABSORPTION OF CARBOHYDRATES

The digestion of carbohydrates starts in the mouth itself and continues into the intestines.

Salivary amylase (ptyalin): The enzyme is capable of acting on polysaccharides like glycogen, starch and dextrins. It acts on the 1,4-glucosidic linkage and liberates maltose. Cl^- and Ca^{++} are required as activators. Optimal pH of the enzyme is 6.9. It is inactivated below pH 5.0. Pepsin also inactivates the enzyme. Hence the action of the enzyme is of a short duration during the momentary stay of the bolus of food in the mouth and for about fifteen minutes thereafter in the stomach. By that time the highly acidic gastric juice containing pepsin is secreted in sufficient amounts to inactivate the amylase.

$$\text{Glycogen or Starch} \xrightarrow[\text{amylase}]{\text{Salivary}} \text{Dextrins (amylo} \rightarrow \text{erythro} \rightarrow \text{achro)} \xrightarrow[\text{amylase}]{\text{Salivary}} \text{Maltose}$$

There is no enzyme in the gastric juice capable of acting on carbohyrates.

Pancreatic amylase (diastase or amylopsin): The action of this enzyme and the conditions of activity are similar to those of the salivary enzyme. The acid chyme from stomach is rendered nearly neutral by the secretion of bicarbonate rich bile and intestinal secretions besides the bicarbonate present in pancreatic juice itself. The long duration of the stay of the food material in the intestines facilitates complete digestion of the starch and other polysaccharides to maltose.

The glucose molecules linked by α -1, 6-linkages at the branching point form isomaltose. This is acted upon by isomaltase to form glucose.

Intestinal Disaccharases (Oligosaccharases):

Several disaccharases and oligosaccharases occur in the intestinal brush border. They can digest maltose, isomaltose, lactose, sucrose and oligosaccharides produced by amylase digeston. They are accordingly called maltase, isomaltase, lactase, sucrase and oligosaccharase. The final products of digestion will be glucose, fructose and galactose.

Genetic deficiency or total absence of some of these enzymes can occur and give rise to defective digestion and absorption of particular disaccharides. Deficiency of lactase activity in infants leads to intolerance to milk and a failure to thrive normally. Cow's milk is tolerated by these children better than mother's milk due to lower lactose content of the former.

Lactase activity is high at birth, but falls rapidly from age two to five years. It is quite low in adults. There appears to be a racial variation in this. While Europeans retain lactase activity in adult life, American Blacks, most Asians and Africans have very low lactase activity in adult life.

Sucrase activity may be low or absent in some individuals.

As a result of the combined action of the various carbohydrases, all the digestible carbohydrates are converted to monosaccharides – mainly glucose and smaller amounts of galactose, fructose and others.

Absorption of carbohydrates: Only monosaccharides are absorbed. Small intestine is almost exclusively the site of absorption. All monosaccharides are not absorbed at the same rate. The sugars appear to be absorbed by a selective mechanism and not by mere diffusion.

If the rate of glucose absorption (called the absorption coefficient) is arbitrarily assigned a value of 100, the other sugars are absorbed at different rates as follows:

Galactose (110) glucose (100) fructose (43). All of them are hexoses with same molecular size. In fact the pentose, arabinose, is absorbed at a much slower rate (9).

Further, glucose is absorbed at the same rate whether it is present in 25% solution or 80% solution in the intestines.

Carrier molecules are present for fructose. Absorption of fructose occurs by facilitated diffusion and is relatively slow and takes place in the direction of the gradient. Glucose and galactose have a common carrier molecule. In this case also, absorption initially is by diffusion along the gradient. As the concentration in the intestinal lumen falls, active transport occurs mediated by Na^+, K^+ –ATPase. Glucose absorption is coupled to sodium absorption. For each molecule of glucose absorbed, two Na^+ are absorbed. The Na^+ is rapidly transported into plasma.

The glycoside '*phlorizin*' blocks glucose entry by blocking the glucose site. It has no action on the Na^+ site.

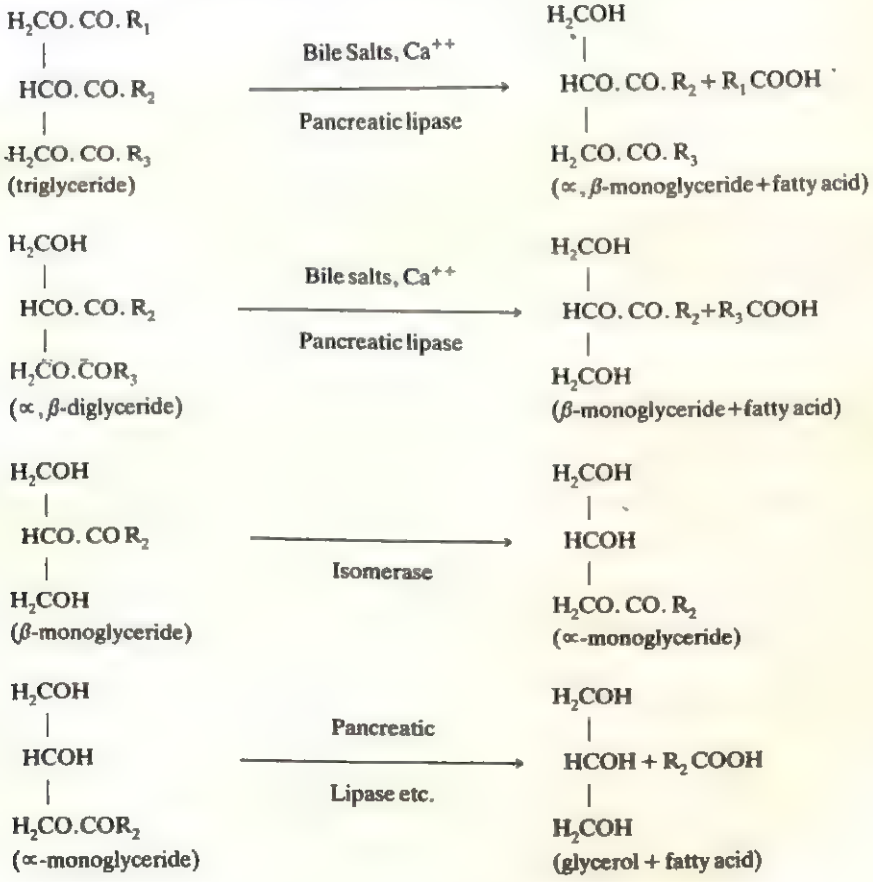
From the epithelial cell, glucose is transported into plasma, again, partly by diffusion (about 25%) and partly by active transport by a carrier system (about 60%). A small amount may be carried back into the lumen of the intestine by the same carrier system.

The active transport of glucose from the intestinal epithelium into plasma (60% of total) is also blocked by phlorizin.

DIGESTION AND ABSORPTION OF LIPIDS

There are no enzymes capable of acting on lipids in the salivary juice or gastric juice. The gastric lipase is of no significance in the adult on account of the highly acid nature of the secretion which renders the enzyme inactive. Hence the digestion of the lipids commences only in the intestines.

Pancreatic lipase: This is the most potent lipolytic enzyme. It is activated by bile salts and its optimal pH lowered to about 6.0 as mentioned earlier. It is also activated by Ca^{++} ions which act mainly by removing the fatty acids formed as insoluble calcium soaps, thus preventing the accumulation of products of the enzyme reaction. The neutralization of gastric chyme by the bicarbonate containing secretions – bile, pancreatic juice and intestinal juice – provides the optimal pH. To provide proper contact between the enzyme and the water-insoluble lipids, bile salts bring about emulsification of the lipid. This emulsification process is further aided by substances like lecithin present in food and partial digestion products of the fat such as monoglycerides. The pancreatic lipase hydrolyzes preferentially the ester linkages on the primary hydroxyl groups of the triglyceride (1 & 3 carbons). The ester group attached to the β carbon (secondary hydroxy group) is not usually hydrolyzed.



Colipase: Pancreatic secretion also contains an enzyme colipase which helps to anchor the lipase to the fat droplet. Acting in association with bile salts, it also lowers the optimal pH of lipase from 8.5 to 6.0, the pH which usually obtains in the contents of the small intestine.

Normally the digestion by pancreatic lipase does not proceed beyond the monoglyceride stage. The intestinal epithelium contains an enzyme which is a monoglyceridase. It is doubtful whether this is secreted into the lumen of the intestine or acts within the epithelial cell. It is capable of hydrolyzing the α -monoglyceride to glycerol and fatty acid.

Lipolysis is a relatively slow process, and, at the end of the few hours usually available for the action of the pancreatic lipase, the triglyceride is only partially hydrolyzed resulting in a mixture of glycerol, fatty acid, mono and diglycerides and some unhydrolyzed triglyceride, all in a finely emulsified state.

Phospholipases: Two phospholipases A & B are said to be secreted in the intestinal juice which hydrolyze phospholipids to liberate the fatty acids. The phosphate and the base are hydrolyzed by phosphatases.

Cholesterol esterase: This is capable of hydrolyzing cholesterol esters in the presence of bile salts to liberate free cholesterol and fatty acids.

Absorption of lipids: According to the present state of knowledge, the following seems to be the most plausible mechanism for fat absorption —

Free fatty acids, mono and diglycerides and the unhydrolyzed triglyceride and phospholipid all form micelles in the presence of bile salts. The formation of the micelles renders them water soluble and creates conditions favourable for further digestion by lipase. The lipids can also be readily absorbed from the micelles by the microvilli or brush border of intestinal epithelium. The process may involve pinocytosis (already described elsewhere) as one of the possible mechanisms.

Short chain and unsaturated fatty acids are absorbed faster and also enhance the rate of absorption of other fats. Long chain fatty acids have the opposite effect. Fats and oils with low melting points (whether of animal or vegetable origin) are more easily digested and absorbed than those with higher melting points. Human milk fat is more easily digested and absorbed than cow's milk fat.

In the intestinal epithelium, the fatty acids are activated and recombine with the mono and diglycerides to form triglycerides. Some amount of phospholipid also is formed.

Cholesterol is absorbed in the small intestine in the presence of bile salts. It is esterified with fatty acid in the intestinal epithelium to form cholesterol ester.

The triglyceride, phospholipid and cholesterol ester aggregate together in the epithelial cell and take up a thin layer of protein covering to form chylomicrons which are small fat globules (around 0.5 μ diameter) covered by a thin lipoprotein layer. These chylomicrons are extruded into lymphatics (lacteals) from the epithelial cell by a process of reverse pinocytosis and are collected into the thoracic duct and from there enter the systemic circulation direct.

However fatty acids of chain lengths below 12 carbons are absorbed by the intestinal epithelium and transported directly (without resynthesis into triglycerides) into portal blood. Some of the phospholipids may also be directly absorbed into portal blood stream.

Much of the absorption of the lipids takes place in the proximal part of the small intestine. The bile salts are not absorbed at this point. They are absorbed in the lower portion to be reexcreted in the bile again (enterohepatic circulation).

Impaired digestion and absorption of lipids

Normally, fecal excretion of fat is about 6 g/day or less than 6% of ingested fat. Failure to digest or absorb fat results in its excretion in larger amounts and is called 'steatorrhoea'. It occurs in the following conditions:

1. Bile salt deficiency: e.g. obstructive jaundice, severe hepatic dysfunction. The stool is clay colored due to absence of stercobilinogen derived from bile pigment. Triglycerides are digested, but the fatty acids are not absorbed. Feces contain increased amounts of lipid, mainly in the form of fatty acids and calcium soaps. Absorption of fat-soluble vitamins is also impaired leading to their deficiency symptoms — particularly vitamin K deficiency.

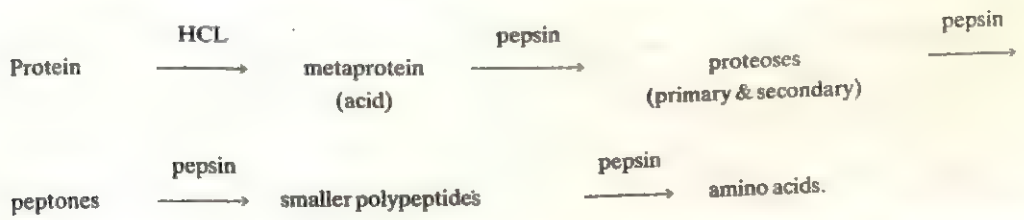
2. Pancreatic deficiency: Feces contains incrased amount of triglycerides. Fat soluble vitamins are absorbed and there are no manifestations of their deficiency.

3. Malabsorption syndrome: In conditions like celiac disease and sprue, not only fat, but also other nutrients and vitamins are not absorbed. This is due to malfunction of the intestinal mucosa.

DIGESTION AND ABSORPTION OF PROTEINS

There is no digestion of protein in the mouth.

Gastric pepsin: Gastric juice contains the enzyme Pepsinogen which is activated to pepsin by H^+ ion. Pepsinogen has a molecular weight of 40,400 and isoelectric pH of only 3.7. Pepsin has a molecular weight of 32,700 and isoelectric pH of only 1.0. Small amounts of pepsin formed will autocatalytically activate the remaining pepsinogen to form pepsin. It has an optimum pH of around 2.0 and is therefore active only in the stomach. It can hydrolyze proteins through stages ultimately to amino acids if allowed sufficient time.



During the relatively short stay of the food in the stomach, the peptic digestion liberates very little of amino acids. Mostly small peptones and polypeptides are produced.

Pepsin is classified as an endopeptidase to indicate that it acts on the central peptide linkages of a protein to split it into small polypeptides. It acts preferentially on peptide bonds involving the –COOH groups of phenylalanine, tyrosine and tryptophan. Exopeptidases, on the other hand, act on the outer peptide linkages to liberate amino acids straightaway (e.g.: carboxy and aminopeptidases).

Pepsin has also a milk curdling property (like rennin) whereby the soluble casein of milk is converted into the insoluble paracasein.

$$\text{Casein} \xrightarrow[Ca^{++}]{\text{rennin or pepsin}} \text{paracasein (calcium paracaseinate, insoluble).}$$

Pancreatic trypsin: Secreted as trypsinogen in the pancreatic juice, it is activated by the enzyme 'enteropeptidase' produced by intesinal mucosa. Small amounts of trypsin formed can autocatalytically activate the remaining trypsinogen. The mechanism of activation involves the removal of a hexapeptide — val-asp-asp-asp-asp-lys — from the zymogen molecule thereby exposing the hitherto masked active site.

Like pepsin, this is also an endopeptidase and under ordinary conditions, produces only small polypeptides from protein molecules. Its optimal pH is nearer neutrality (pH 7.0 to 8.0). Trypsin has particular action on basic proteins like protamines and histones. Pepsin has little action on these.

Trypsin preferentially attacks the peptide linkages involving the carboxyl group of arginine or lysine.

Chymotrypsin (pancreas): Secreted in the inactive form as chymotrypsinogen, it is activated by trypsin. It is also an endopeptidase like trypsin and pepsin. It has an optimum pH of 7.0 to 8.0. The amino acid sequence of chymotrypsin was completely worked out. It has 246 amino acids. It is activated by trypsin by cleavage of a bond between arginine and isoleucine.

It preferentially attacks the peptide linkages involving phenylalanine, tyrosine and tryptophan.

Carboxypeptidase and Aminopeptidase: These are exopeptidases acting on the peptide linkage next to the free carboxylic and free amino groups respectively. While carboxypeptidase is present in pancreatic juice, aminopeptidase is present in intestinal secretion. They have an optimal pH near neutrality (7.4) and by their action on the small polypeptides liberated by the three endopeptidases discussed above, they form free amino acids.

Dipeptidase: This is another enzyme in the intestinal secretion and as the name indicates, it hydrolyzes dipeptides to liberate the two amino acids.

Absorption of amino acids and oligopeptides

Four distinct carrier systems exist in the intestinal epithelial cells for the transport of (1) neutral amino acids, (2) basic amino acids, (3) acidic amino acids and (4) glycine and imino acids (proline and hydroxyproline). Genetic deficiencies in any of these carrier systems will involve both intestinal absorption as well as renal tubular reabsorption. The carrier systems operate along with Na^+, K^+-ATPase. Absorption of amino acids into the mucosal cell from the lumen is associated with absorption of Na^+ from the serosal surface. While the amino acid transport is against gradient (uphill), the absorption of Na^+ is along gradient (downhill).

Small oligopeptides are also absorbed by separate carrier systems and are hydrolyzed to amino acids in the mucosal cell.

While the absorption of protein is almost entirely as amino acids, there is evidence to indicate that small intestine has the ability to absorb minute amounts of undigested protein also. This explains the allergic reactions people show for particular type of proteins — milk, wheat (gluten) and others. Only intact proteins are antigenic. Amino acids do not stimulate antibody production.

The hormonal regulation of gastrointestinal secretion is covered under "*Hormones*" and may be consulted at this stage.

11

CHANGES OCCURRING IN THE LARGE INTESTINES

FERMENTATION, PUTREFACTION AND FECES FORMATION

The gastrointestinal tract of the new born infant is sterile. Within hours after birth, the microorganisms from the environment gain entry and make permanent abode in the large intestine chiefly and to a lesser degree in the rest of the gastrointestinal tract. They are relatively few or absent in the highly acidic region of the stomach. The activity of these organisms is helpful in the herbivorous animals in digesting cellulose (the enzyme cellulase is absent in the digestive secretions of the animals) and in all the higher animals including man in synthesizing several of the B-complex group of vitamins which are absorbed and utilized by the host organism. They also produce several harmful products by their action on the intestinal contents, the absorption of which can give rise to toxic symptoms. These changes brought about on the food materials by the bacteria are mostly non-oxidative reactions — usually reductive in nature — and are termed 'fermentation' in case of carbohydrates and lipids and 'putrefaction' in case of proteins.

Action on carbohydrates and lipids (fermentation): As a result of bacterial action, organic acids like acetic, butyric, and lactic and gases like methane, carbondioxide and hydrogen are produced. The choline present in lecithin may be converted to a toxic substance named 'neurine'.

$$\underset{\text{Choline}}{(CH_3)_3N^+CH_2CH_2OH\ OH^-} \longrightarrow \underset{\text{Neurine}}{(CH_3)_3N^+CH=CH_2\ OH^-}$$

Otherwise most of the substances produced by fermentation are non-toxic.

Actions on proteins and amino acids (putrefaction): The reactions include hydrolysis of proteins and polypeptides, decarboxylation and deamination of the amino acids (non-oxidative) yielding the corresponding amines or fatty acids and products like H_2S and mercaptans from sulfur containing amino acids.

$$\underset{\text{(Amino acid)}}{R.CHNH_2COOH} \xrightarrow{-NH_2} R\,CH_2COOH\ \text{(Fatty acid)}$$

$$\underset{\text{(Amino acid)}}{R.CHNH_2COOH} \xrightarrow[-CO_2]{} R\,CH_2NH_2\ \text{(Amine)}$$

The amines produced from some of the amino acids are quite toxic, if absorbed. They are potent vasopressors and constrictors of smooth muscle.

E.g.: Tyrosine $-CO_2$ ⟶ Tyramine

Tryptophan $-CO_2$ ⟶ Tryptamine

Histidine $-CO_2$ ⟶ Histamine

Cysteine $-CO_2$ ⟶ Ethyl mercaptan

Lysine $-CO_2$ ⟶ Cadaverine

Arginine ⟶ Ornithine $-CO_2$ ⟶ Putrescine

The aromatic benzene ring of the amino acids, phenyl alanine and tyrosine, is converted, after removal of the side chain, to phenols and cresols. In the case of tryptophan, the products are indole and skatole. The potassium salt of indoxyl sulphate (derived from indole) is absorbed and excreted in urine. Its presence in appreciable amounts in urine indicates excessive putrefaction in the intestines and is suggestive of intestinal stasis or obstruction.

The bile pigments undergo the changes described in the preceding chapter and get converted to stercobilinogen by bacterial action.

Some of the reactions involved in the above processes are outlined below:

$CH_2SH\,CHNH_2\,COOH$ (Cysteine)

$-CO_2$ ⟶ $HS\,CH_2\,CH_2\,NH_2$ Ethyl mercaptan

$-CO_2$ ⟶ $CH_4 + H_2S$

$NH_2\,CH_2(CH_2)_3\,CHNH_2\,COOH$ Lysine $\xrightarrow{-CO_2}$ $NH_2(CH_2)_5NH_2$ Cadaverine

$NH_2\,CH_2(CH_2)_2\,CHNH_2\,COOH$ Ornithine $\xrightarrow{-CO_2}$ $NH_2(CH_2)_4NH_2$ Putrescine

Formation and composition of feces: Throughout the course of the passage of the digested food materials (chyme) through the small bowel, along with digested materials, enough water also is absorbed, leaving a semi-liquid, undigested, unabsorbed material which enters the colon. The colon secretes a slightly alkaline mucinous fluid which serves as a lubricant and is devoid of any enzymes. In the colon the surplus water is absorbed. Any nutrients not absorbed by the small intestine will be passed unabsorbed by the large gut. Due to removal of water in the large bowel, the contents become semisolid and gain the normal consistency of formed feces.

The feces contain about 75% water, 20% organic solids and 5% ash (inorganic). The organic material consists of the undigested and unabsorbed food material like cellulose, desquamated mucosal cells and billions of bacteria. In fact the bacteria account for 25% of the dry weight of the feces. The inorganic material is mainly insoluble salts — phosphates and oxalates of calcium and iron. The dark brown color of the feces is on account of the pigments bilifuscin and stercobilin. The foul odor is on account of indole and skatole mainly and to a lesser extent due to H_2S and mercaptans — all results of protein putrefaction. Nitrogen, CH_4 and CO_2 are the main gases. In case there is stasis H_2 and H_2S also accumulate.

A healthy adult excretes about 100–200 grams of feces per day. This increases if large amount of vegetables are consumed in diet (due to high cellulose content). The pH is near neutral — 7.0 to 7.5. A predominantly carbohydrate diet will produce excessive fermentation and may lead to a more acid pH.

The lipid content of the feces normally is around 5-15% of the dry weight of the feces. This is composed largely of plant sterols, reduction products of cholesterol, neutral fat, fatty acids, and soaps. If large amounts of fat are excreted, the condition is called steatorrhoea.

References (for chapters 10 & 11):

Babkin, B.P.: Secretory Mechanism of Digestive Glands, 2nd. ed., Hoeber, N.Y. 1950.

Conway, E.J.: The Biochemistry of Gastric Acid Secretion, Thomas, Springfield, ILL., 1953.

Gray, C.H.: Bile Pigments in Health and Disease., Thomas, Springfield, ILL., 1961.

James, A.H.: The Physiology of Gastric Secretion, Arnold, London, 1957.

Verzar, F.: Absorption from the Intestine., Longmans., N.Y., 1936.

Wilson, T.H.: Intestinal Absorption, Saunders, Philadelphia, 1962.

Wiseman, G.: Absorption from the Intestine, Acad. Pr., N.Y., 1964.

Wolf, S. and Wolf, H.G.: Human Gastric Function., Oxford Univ. Pr., 1947.

12

DETOXICATION MECHANISM OR METABOLISM OF FOREIGN COMPOUNDS

SEVERAL unwanted and harmful substances get entry into the body either by absorption from the gastrointestinal tract (*e.g.:* products of putrefaction and fermentation or drugs administered orally) or by the parenteral route. Some of the physiological substances normally produced in the body (*e.g.:* hormones) also require to be eliminated regularly to prevent accumulation and prolonged and cumulative action. The mechanisms which convert the substances into readily excretable and in most cases into less harmful products are known as detoxication mechanisms. Since not all the products of such conversion are less toxic (a few are actually more toxic), metabolism of foreign compounds is a more accurate term. The foreign substances undergo some preliminary changes which are not different from the metabolic changes occurring to physiological substances. These include (1) oxidation, (2) reduction and (3) hydrolysis. The products in most cases are then subjected to a final process (4) 'synthesis' or 'conjugation' or coupling with some substance in the body which will render it to a form suitable for excretion. It is then eliminated through the urine. Most of the detoxicating mechanisms occur in the liver which is therefore an important organ for this function. A brief description of the process with examples is given below:-

1.Oxidation: (i) Alcohols are oxidized to corresponding acids:

Eg. $CH_3OH \longrightarrow H\,COOH$
methanol — formic acid

Benzyl alcohol — Benzoic acid

(ii) Aromatic hydrocarbons are oxidized to phenols.

Benzene — phenol

2. Reduction: (i) Aldehydes are reduced to alcohols:

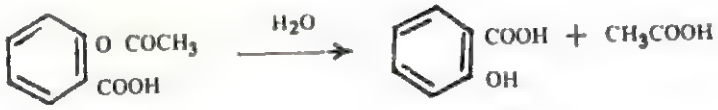
CCl_3CHO (chloral) ⟶ CCl_3CH_2OH (Trichloroethanol)

(ii) Aromatic nitro compounds are reduced to corresponding amines:

3. Hydrolysis: Drugs like procaine and acetyl salicylic acid and cardiac glycosides like digitalin undergo hydrolysis.

$NH_2C_6H_4COO\,CH_2CH_2N\,(C_2H_5)_2 + H_2O$
Procaine

⟶ $NH_2C_6H_4COOH$ (p-aminobenzoic acid) + $HOCH_2CH_2N(C_2H_5)_2$ (diethylaminoethanol)

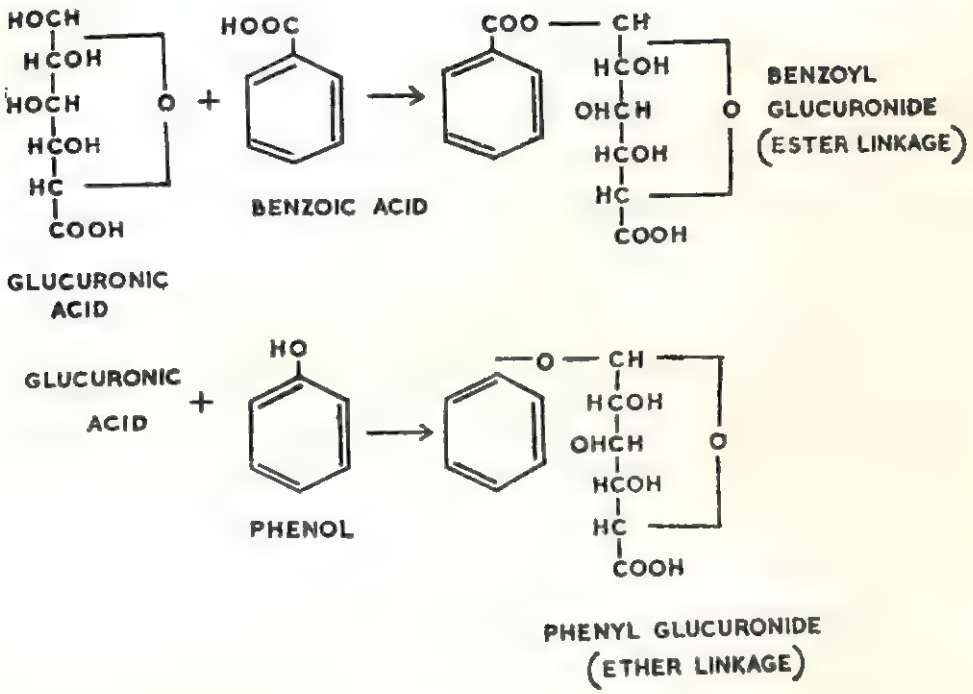


Acetyl salicylic acid — salicylic acid

4. Conjugation: The foreign substances as such or after suitable preparation by one or other of the processes of oxidation, reduction and hydrolysis are usually conjugated with another substance before they are excreted.

The following are some examples —

(i) *Glucuronic acid:* Aromatic acids (*e.g.:* benzoic acid) and phenols are conjugated with glucuronic acid. The glucuronic acid is derived from uridine diphosphate glucuronic acid. The drug chloramphenical, and the bile pigments are among the important substances conjugated with glucuronic acid. Derivatives of steroid hormones also are conjugated with glucuronic acid before excretion.



(ii) *Active Sulfate* is used to conjugate phenolic compounds.

The derivatives are called ethereal sulfates. An increase of their amount in urine signifies excessive intestinal putrefaction or stasis. Adrenal cortical hormones are also excreted after conjugation with sulfurice acid.

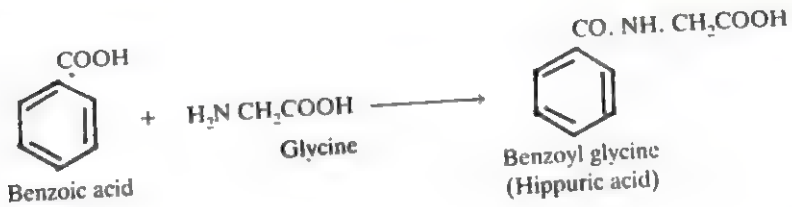
Phenyl sulfuric acid

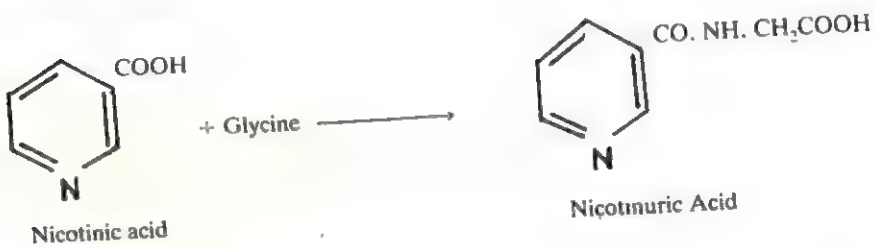
indoxyl Indoxyl sulfuric acid

Yeast and mammalian liver contain enzymes that can activate inorganic sulfate by adding it to 3-phosphoadenosine-5-phosphate. Active sulfate has the following structure– Adenine-3-phosphoribose-5-phosphosulfate.

Incorporation of sulfate into sulfated mucopolysaccharides and conjugation of steroid hormones and others with sulfate are brought about after preliminary activation of sulfate.

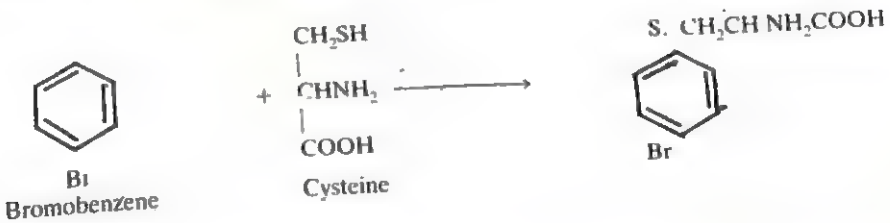
(iii) *Glycine* is used to conjugate aromatic acids, cholic acid and also nicotinic acid.





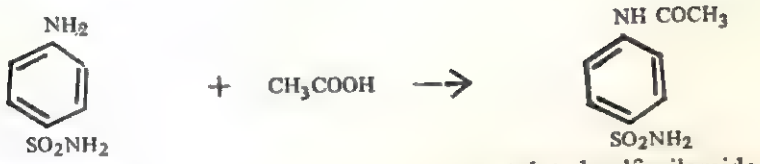
The formation of bile acid (glycocholic acid) from cholic acid is discussed elsewhere.

(iv) *Cysteine* is used in the conjugation of certain aromatic compounds like benzene and halogenated ring compounds like bromobenzene.



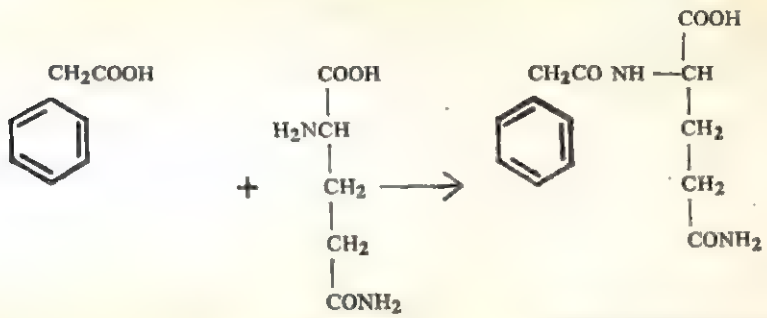
The amino group is later acetylated to form what are known as mercapturic acids.

(v) *Acetic acid* is used to conjugate with aromatic amino compounds like sulfanilamide.



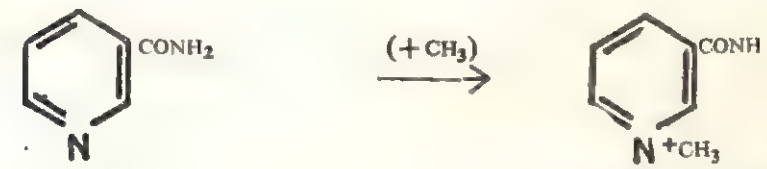
sulfanilamide — Acetylated sulfanilamide

(vi) *Glutamine* is conjugated with phenylacetic acid to form phenylacetylglutamine.



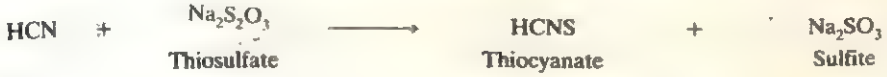
Glutamine — Phenylacetyl glutamine

(vii) *Methyl* groups from active methionine (S ~ adenosyl methionine) are used for conjugation of certain pyridine and other heterocyclic nitrogen containing compounds like nicotinamide.



Nicotinamide — N–Methylnicotinamide

(viii) Interaction of highly toxic cyanides with thiosulfate to form the relatively nontoxic thiocyanates is also included under conjugation reactions.



The enzyme which converts cyanide to thiocyanate is called 'rhodanase'.

References:

Williams, R.T.: Detoxication Mechanism, 2nd Ed, Wiley, N.Y., 1959.

13
INTERMEDIARY METABOLISM

Introduction: The series of changes that a substance undergoes after absorption from the gastrointestinal tract whereby it is used for synthesis of some of the tissue components or is broken down or otherwise altered and eliminated from the body through urine, feces, sweat or respiration are referred to as the 'intermediate metabolism' or simply as 'metabolism' of the substance. The processes by which it is used in the synthesis of tissue components are referred to as 'anabolism' and the processes by which it is broken down into simpler products are referred to as 'catabolism'. Anabolic reactions require energy to be supplied whereas catabolic reactions liberate energy. In the normal adult maintaining constant weight, the two sets of reactions balance each other. There is a constant break down of the different components of the tissues and a constant resynthesis so that at any given time the total amount is the same. The energy for the anabolic reactions as well as the energy required for the different activities of the organism such as conduction of nerve impluses and muscular contraction is supplied by the catabolism of the absorbed food materials. If food is not ingested for a few days, this energy requirement is met by catabolism of tissues leading to a wasting of tissues and loss of weight.

The metabolic processes in both anabolic and catabolic direction are finely regulated by nervous and hormonal control, a study of which is also essential for the proper understanding of metabolism.

METHODS OF STUDY

The following are some of the methods used in the study of metabolism:

1. Analysis of blood and tissues: To find the changes undergone by a substance, a sample of blood or of the tissue where the substance is being metabolized can be taken before and after the substance is administered, and analyzed. Any changes in the composition have to be cautiously interpreted in relation to the metabolism of the substance under study. Eg. When glucose is administered, the glycogen content of muscle and liver increases with a simultaneous fall of glucose levels in the initially raised blood glucose. This can be interpreted to mean that the liver and muscle have taken up the glucose from blood and convereted it to glycogen.

2. Analysis of excreta: Usually urine is analyzed. The quantity of urinary nitrogen excreted in a day will enable the estimation of the amount of protein catabolized during that period. Each gram of urinary nitrogen indicates 6.25 grams of protein catabolized.

In a diabetic individual or in an experimental animal where glucose reabsorption by the renal tubule is prevented by poisoning with iodoacetate or phlorrhizin, the feeding of different substances and the estimation of urinary glucose will give information as to how much of the substance is convertible to glucose in the body.

3. Respiratory exchange: The ratio of the quantity of carbon dioxide eliminated to the oxygen utilized (CO_2/O_2) in a given time is known as the respiratory quotient or R.Q. The R.Q. is 1.0 if carbohydrates are solely being metabolized, 0.7 if fats are solely metabolized and 0.80 for proteins. The R.Q. will thus enable in having an insight into the proportion of the three principal constituents metabolized.

4. Injecting endocrine preparations or removing the endocrine glands: The action of various hormones which influence metabolism can be studied by studying the effects of removal of each of the glands. All the effects produced by the removal will be relieved, if to such an animal, a purified preparation of the hormone is administered, eg. pancreatectomy produces symptoms similar to diabetes mellitus which can be relieved by injecting insulin. Instead of removing the gland, a poison which specifically affects the endocrine producing cells can be used. Thus, alloxan which destroys β-cell of the pancreas, will produce almost similar changes as removal of pancreas.

5. Perfusion of viscera: The perfusion can be done in situ with the viscera still in the body or after isolation and removal from the body. The substance under study is perfused through the arterial supply to the viscera and the blood or perfusate from the vein is collected and analyzed. The tissue itself can be analyzed at the end of the experiment. Perfusion of liver with amino acids may lead to their deamination and the perfusate will contain the corresponding α-keto acids.

6. Tissue slice technique: This was perfected by Warburg. Extremely thin slices (almost unicellular in thickness) can be cut using suitable slicing techniques. Slices cut from a freshly collected liver tissue can be incubated in a suitable medium of saline and nutrients and after a given time the changes undergone by a particular nutrient can be studied by analysis of the medium. The respiratory exchange (O_2 intake and CO_2 output) can also be studied in a suitable apparatus known as Barcroft-Warburg manometric apparatus. Studies on the metabolism of liver and brain are usually performed using such apparatus.

7. Homogenate technique: Instead of intact viscera or tissue slices where the cellular organization is intact, the tissue may be broken up by grinding it with sand or in a homogenizer and the material suspended in saline or suitable medium known as the 'homogenate' can be used for study. The homogenate may also be separated by

ultracentrifugation into the various subcellular components such as nuclei, mitochondria, ribosomes and cytoplasmic supernatant and the location of the enzymes and their action can be studied in each of the fractions separately. Such studies have revealed the occurrence of the reactions of glycolysis in the cytoplasm, the reactions of citric acid cycle and oxidative phosphorylation in the mitochondria and protein synthetic reactions in the ribosomes.

8. Enzyme studies: Since all the metabolic reactions are brought about ultimately by enzymes, and since many enzymes are now available in a pure state, individual enzyme reactions can be studied to get the overall sequence of events.

9. Use of enzyme inhibitors: Many of the reactions proceed as chain reactions and it is difficult to study the intermediate substances formed. Eg. glucose or glycogen is rapidly converted to pyruvic acid or lactic acid by action of a series of enzymes. If one of the enzymes involved in the chain of reactions is inhibited by a poison the preceding reactions in the chain can be studied; eg. iodoacetate inhibits the sulfhydryl enzyme glyeraldehyde-3-phosphate dehydrogenase. Various hexose phosphates accumulate and can be studied.

10. Inborn errors of metabolism: Nature occasionally provides similar opportunities in certain diseases known as 'molecular diseases' or 'inborn errors of metabolism'. One of the enzymes required in the metabolism is absent from birth giving rise to accumulation and excretion of the earlier intermediates formed and thus enabling a study of the metabolic process of that substance; eg: alkaptonuria and cystinuria in the study of metabolism of homogentisic acid and sulfur containing amino acids.

11. Studies with microorganisms: Microorganisms can be grown on purified media. Since they multiply at a rapid rate, the changes produced in the media and the metabolic processes can be studied in detail. It is also possible to produce mutant strains of the microorganisms and study the effects of the lack or addition of an enzyme or a metabolite. Much of the recent advances in genetics, we owe to studies on the microorganism E.coli.

12. Use of radioactive and mass isotopes: In more recent times, the availability of isotopes and instruments for the detection of radioactive as well as mass isotopes, have aided greatly in confirming some of the earlier findings arrived at by simpler methods and in making further advances in several hitherto unexplored fields as well. Substances can be prepared incorporating the isotope instead of the natural element. Such substances are said to be 'tagged' or 'labelled' eg: Glucose can be prepared containing C_{14} in one or more of its carbons instead of the normal C_{12}. The tagged substances can be administered to the intact animal and the changes undergone by the substance can be tracked by analyzing the substances which contain the 'label'.

	Natural element	**Isotope used in studies**
1. Hydrogen	$_1H^1$	Deuterium $_1H^2$ Tritium $_1H^3$
2. Carbon	$_6C^{12}$	$_6C^{11}$, $_6C^{13}$, $_6C^{14}$
3. Nitrogen	$_7N^{14}$	$_7N^{15}$
4. Oxygen	$_8O^{16}$	$_8O^{18}$
5. Sodium	$_{11}Na^{23}$	$_{11}Na^{24}$

6. Phosphorus	$_{15}P^{31}$	$_{15}P^{32}$
7. Sulfur	$_{16}S^{32}$	$_{16}S^{35}$
8. Chlorine	$_{17}CL^{35}$	$_{17}CL^{36}$
9. Potassium	$_{19}K^{39}$	$_{19}K^{42}$
10. Calcium	$_{20}Ca^{40}$	$_{20}Ca^{45}$
11. Iron	$_{26}Fe^{56}$	$_{26}Fe^{59}$
12. Cobalt	$_{27}Co^{59}$	$_{27}Co^{60}$
13. Iodine	$_{53}I^{127}$	$_{53}I^{131}$

By using such methods it was found that in glycoysis the third and fourth carbons of glucose are oxidized first, whereas in hexose monophosphate pathway, the first carbon is oxidized earliest.

BIOENERGETICS

The energy required for anabolism and the energy liberated in catabolism are all on account of chemical changes occurring in the organism. Every chemical substance has a certain amount of energy built into it which is the energy of the chemical bonds holding the atoms together. This is described as the 'free energy' of that substance. To synthesize the compound from its elements this much energy has to be expended. Conversely, if the compound is broken down to its elements, this much energy is released.

Exergonic and endergonic reactions: When two substances are capable of interacting to liberate some products as represented by the equation:

$$A + B \longrightarrow C + D$$

(*i*) The reaction will proceed spontaneously with the liberation of energy if the free energy of A and B (reactants) is greater than the free energy of C and D (products). Such reactions are said to be exergonic (energy liberating). The chemical energy so released can be used to do work.

(*ii*) If the free energy of A and B (reactants) is less than that of C and D (products), then the reaction cannot proceed to the right unless energy is supplied from other sources. Such reactions are said to be endergonic (energy requiring).

The former represent catabolic reactions and the latter anabolic reactions.

Source of energy: The ultimate source of energy for all living matter is the sunlight. The plant kingdom absorbs the energy from the sunlight, particularly from the ultraviolet radiations and utilizes it to synthesize the carbohydrate, starch, a polysaccharide of glucose, from the atmospheric CO_2 and H_2O (photosynthesis).

$$6CO_2 + 6H_2O + 708{,}000 \text{ calories} \longrightarrow C_6H_{12}O_6 + 6O_2$$

The free energy change of the reaction is 708,000 calories and this is supplied by sunlight to bring about the reaction. When the reaction is reversed, an equivalent amount of energy is released.

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + 688{,}000 \text{ calories.}$$

The plant will utilize the energy originally trapped in the form of carbohydrate for building up the rest of the components of its structure. The herbivorous animals directly depend on the plant materials and the carnivorous animals also indirectly depend on the plant material.

Release of energy: The oxidation of glucose directly in an atmosphere of O_2 will release, as seen earlier, 688,000 calories of energy. This is liberated rapidly and explosively and is dissipated away as heat. If it were to be used for bringing about other chemical reactions in the tissues or for doing work, there should be methods available in the organism which prevent such explosive liberation of energy. This is, in fact, so. The oxidation of glucose does not proceed directly as depicted in the above reaction, but it is channelled through a stepwise process where the several steps in the exergonic reaction are coupled to other synthetic reactions (endergonic) which take up the energy of glucose oxidation and store it in themselves as chemical energy. Even so, nearly 50% of the energy is still dissipated. The chemical energy released in glucose catabolism is mainly used in the addition of phosphate to the nucleotide structure, adenosine diphosphate, by a pyrophosphate linkage to form adenosine triphosphate (abbreviated as ATP).

$$C_6H_{12}O_6 + 6O_2 + 38 \text{ ADP} + 38H_3PO_4$$

$$\longrightarrow 6CO_2 + 44H_2O + 38 \text{ ATP} + 384{,}000 \text{ calories.}$$

The 38 ATP can be hydrolyzed subsequently, as required, to yield again 38 ADP and 38 phosphate molecules and energy of over 300,000 calories which can be used to drive an endergonic reaction forward (synthesis or work).

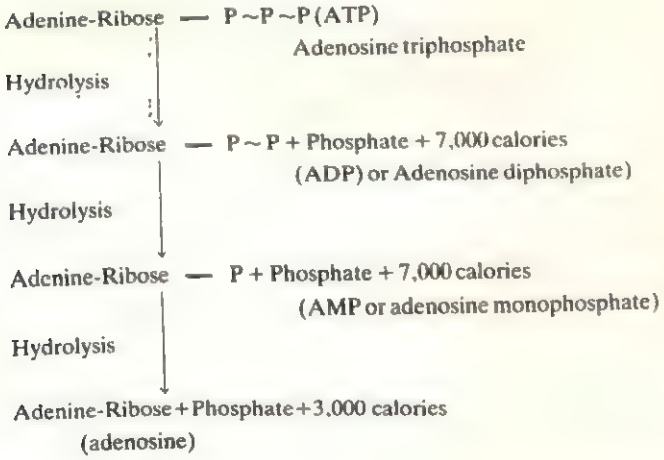
The mechanism of coupling oxidation of glucose or any other substrate with the phosphorylation of ADP to produce ATP is known as *oxidative phosphorylation*.

Oxidative phosphorylation: The coupling of respiration to phosphorylation was first hinted by Engelhardt in 1930 in the Soviet Union. But clear experimental evidence was shown by Kalckar of Denmark and Belitser in the Soviet Union only in 1937. E.P. Kennedy and A.L. Lehninger (1948) showed that oxidative phosphorylation occured in the mitochondrial fraction of the cell. Lehninger also showed that for every atom of oxygen utilized in the respiratory chain, three ATPs can be generated (P:0 ratio = 3).

ADP and inorganic phosphate are required for the normal functioning of the respiratory chain. In fact, the rate of respiration of the mitochondria is regulated by the quantity of ATP present. The folding of the inner membrane and the cristae become accentuated in the presence of ADP. There is a resultant decrease in the volume of the matrix.

E. Racker has shown that the head piece of the elementary body is involved in coupling phosphorylation to electron transport and the enzymes concerned with electron transport are all located in the inner mitochondrial membrane. ATP is but one of a group of compounds where a particular chemical bond carries more free energy than other similar bonds. The hydrolysis of ATP in stages will clarify the concept of an energy rich bond.

High-Energy Phosphates or Energy-Rich Phosphates: ATP was first discovered in muscle extracts by C. Fiske and Y. Subbarow in the United States of America and by K. Lohmann in Germany independently in 1929.The work of Warburg. Meyerhoff, Kalckar, Engelhardt and the Coris lead to the elucidation of the role of ATP in metabolism. Lipmann (1941) presented a detailed hypothesis of its functions as an energy rich phosphate. At neutral pH, the terminal two phosphate groups are in an ionized form and form a complex with the divalent Mg^{++}. In most enzyme reactions where ATP participates as phosphate donor, its active form is $MgATP^{=}$ complex.



Thus in the hydrolysis of each of the terminal two phosphate groups (which are each linked to the rest of the molecule by a pyrophosphate linkage) about 7,000 calories of energy is liberated. The hydrolysis of the first phosphate, which is linked by an ester linkage to ribose, releases, on the other hand, only 3,000 calories. To distinguish the terminal two pyrophosphate bonds from the first ester linkage, the terminal phosphate bonds are called energy rich or high energy phosphate bonds and are represented in the structural formulae thus (~) instead of by a straight line (–).

There are several such energy rich compounds formed during metabolism. Some are listed below:

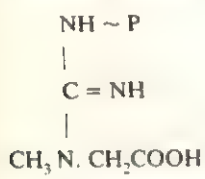
1. *ATP* (Adenosine triphosphate): The role of ATP as a high energy phosphate has been mentioned earlier. When there is a shortage of ATP and oxidative phosphorylation is inadequate, ATP can be formed from ADP by the following reaction:

$$2\ ADP \longrightarrow ATP + AMP$$

This reaction is catalyzed by the enzyme *'adenylate kinase'* which is widely distributed in all tissues.

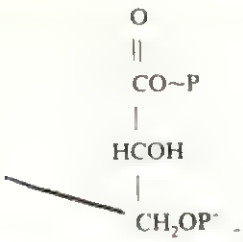
2. *Creatine phosphate:* This is the main storage form of energy in muscle. ATP is present only in small amounts. When this is used up in muscular contraction, it is replenished by trnasfer of energy rich phosphate from creatine phosphate.

$$\text{Creatine Phosphate} + ADP \longrightarrow \text{Creatine} + ATP$$

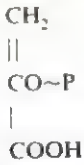


In the invertebrate muscle, arginine phosphate is the storage form of energy.

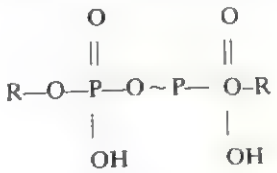
3. 1,3-*Diphosphoglyceric acid.* This is formed during glycolytic breakdown of glucose. Only the phosphate bond attached to the carboxylic group is energy rich.



4. *Phosphoenol pyruvate:* This is also formed during glycolytic breakdown of glucose.



5. *Pyrophosphate in general is energy rich:*



6. *Acetyl coenzyme A:* $CH_3\ CO \sim \grave{S}CO\ A$

This is also known as active acetate. The linkage here is not phosphate linkage but a thioester linkage between - COOH and -SH.

7. Other energy-rich compounds:

Adenosine 3',5' cyclic monophosphate (cyclic AMP or cAMP) and guanosine 3',5' cyclic monophosphate (cGMP) are also high energy compounds. They occur almost in all cells and are produced from ATP and GTP by the action of enzymes adenylate cyclase and guanylate cyclase. They do not have any role in energy storage but play an important role in the regulation of enzyme activities.

Acyl thioesters, like fatty acyl esters of coenzyme-A are also high energy compounds.

Aminoacyl esters of the ribose in tRNA, sulfonium compounds like S-adenosyl methionine (active methionine) and acyl esters of carnitine are also examples of high energy bond compounds.

Energy release during hydrolysis of the phosphate bond as – $\triangle G^{\circ}$ at ph 7.0

Phosphoenol pyruvate	...	13.0	ATP, inner bond	...	8.5
Cyclic AMP...	...	11.9	ATP terminal bond	...	8.4
Acetyl phosphate	...	10.5	Acetyl CoA	...	7.7
S-Adenosly methionine	...	10.0	Palmityl carnitine	...	7.7
Phosphocreatine	...	9.0	Glucose-1-phosphate	...	5.0
			Glycerol-3-phosphate	...	3.0

BIOLOGICAL OXIDATION – REDUCTIONS

As considered in the two earlier sections, energy is obtained by the organism by oxidation of glucose or other substrates. No oxidation can proceed unless there is a simultaneous reduction of some other compound. The two are coupled, oxidation-reduction. To facilitate the stepwise oxidation of substances like glucose in the tissues, it is necessary to have a number of substances or systems which can undergo simultaneous reduction. There should also be mechanisms to reoxidize the reduced systems, so that they can again participate in the original reaction. The terms 'oxidation' and 'reduction' are used in the broadest sense. Oxidation includes

(i) addition of oxygen

Eg: $CH_3CHO + \frac{1}{2}O_2 \longrightarrow CH_3COOH$

(ii) removal of hydrogen

eg: $CH_3CH_2OH - 2H \longrightarrow CH_3CHO$ and

(iii) removal of an electron

$Fe^{++} - e \longrightarrow Fe^{+++}$

(Ferrous iron) (Ferric iron)

In fact, in all the above examples, there is one thing in common–loss of electrons. In (iii) it is quite obvious. In (i) the oxygen atom shares the electrons of H and thus reduces partially its electron content and in (ii) the hydrogen atoms remove the two electrons previously shared by the oxygen atom.

In reduction the opposite changes are involved. Hence a substance A will be able to oxidize another substance B, if it can take up the electrons from B. An oxidizing substance

can therefore take up and hold electrons to itself while a reducing substance can part with electrons.

This is only with respect to the two substances considered. The substances A and B may both be reduced by a third substance C if C can give up electrons to A and B readily. To be able to predict which system will oxidize or reduce which other system, it is necessary to know what is described as the oxidation-reduction potential of that system, abbreviated as the 'redox' potential of that system. Without going into all the details of how these are arrived at, the redox potentials of the biologically important systems are tabulated below. It may be noted that the systems mostly belong to the compounds we are familiar with as coenzymes while studying the vitamins, particularly the B-complex group.

Redox system	*Redox potential (E_o^1 (volts) Eh)*
$\frac{1}{2}O_2/H_2O$	+0.82
cytochrome a/cytochrome a (Fe^{+++}) /(Fe^{++})	+0.29
cytochrome c/cytochrome c (Fe^{+++}) /(Fe^{++})	+0.22
cytochrome b/cytochrome b (Fe^{+++}) /(Fe^{++})	+0.08
Flavoprotein FP/FPH_2	–0.12
$NAD^+/NAD.H+H^+$	–0.32

The system with the higher redox potential (more positive or less negative) can readily take up electrons from the one with the lower redox potential (the less positive or more negative). Thus in the table above, each system can take up electrons from (oxidize) the systems below and lose electrons to (reduce) the systems above.

While transfer of electrons from the most negative system ($NAD^+/NAD.H+H^+$) to the most positive ($\frac{1}{2}O_2/H_2O$) will liberate all the energy at one time in an explosive manner (like a water fall), their transfer in a stepwise manner through all the intermediate systems will enables a slower release of energy and its capture (as when a dam is constructed and the water is allowed to turn a turbine) to synthesize energy-rich compounds. This is the principle involved in biological oxidations and oxidative phosphorylation. The chain of electron transfers involved is described as the *electron transport chain or the respiratory chain* of reactions. A complete such chain is depicted in Fig. 13-1.

During the oxidation of the metabolite AH_2 to A the 2H have to be ultimately oxidized to H_2O. This is done stepwise by passing on the hydrogen successively to coenzymes NAD (or NADP as the case be), flavoprotein (FAD or FMN) and to coenzyme Q. At this stage,

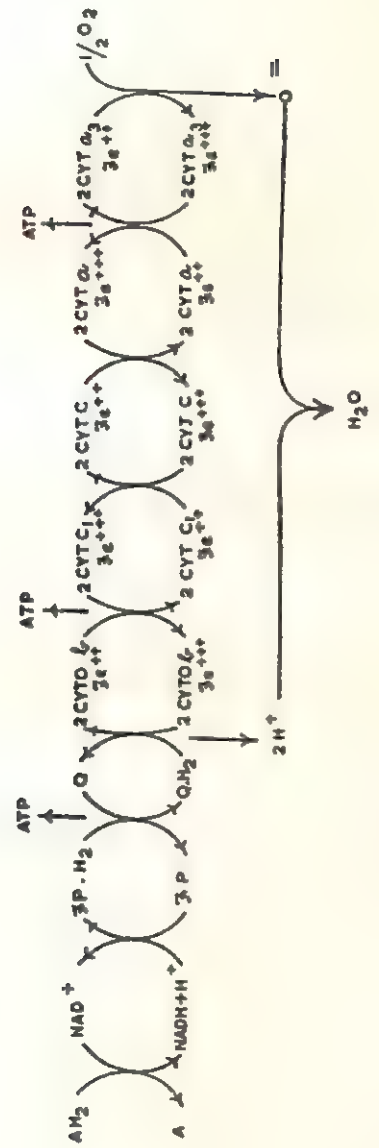


Fig. 13-1. Electron Transport Chain

only the electron from the hydrogen is taken up leaving the ionic form of H^+ in the medium. The electron is transported through the various components of the cytochrome system-b, c_1, c, a and a_3 and finally passed on to elemental oxygen which is thereby converted to ionic $O^=$ and can readily combine with $2H^+$ to form a molecule of H_2O. The energy from the oxidation of hydrogen is accumulated in each step and is liberated when it reaches a threshold value in three stages as indicated in Fig. 13-1 to form three molecules of ATP from ADP:

(i) between flavin system and coenzyme Q

(It is possible that more than one flavoprotein participate in the transfer of hydrogen from the NAD system to coenzyme Q. The generation of ATP seems to occur in the transfer of hydrogen from the flavoprotein of lower redox potential to that of the higher redox potential. This step has ben omitted in fig. 13-1).

(ii) between cytochrome b and c_1 systems and

(iii) between cytochrome a and a_3 systems.

Not all oxidations need proceed through the entire chain. There are some which by-pass the earlier NAD system; eg succinic dehydrogenase. In this the hydrogen is taken up by the flavoprotein of the enzyme direct without intervention of NAD. Hence only two ATP are generated.

Role of mitochondria: The electron transport chain is located in the protein component of the inner membrane that forms the cristae. The enzymes of the citric acid cycle and of beta oxidation, the two major oxidative pathways of metabolism, are located in the matrix enclosed by the inner membrane.

Mechanism of Oxidative Phosphorylation: Several hypotheses are proposed.

1. *Chemical Coupling:* During electron transfer reactions a high energy intermediate compound is formed and the energy from this is utilized to form ATP from ADP.

$$\text{eg. } AH_2 + B + C \longleftrightarrow A \sim C + BH_2$$

$$A \sim C + ADP + P \longleftrightarrow A + C + ATP$$

This is analogous to the substrate phosphorylation that occurs in the oxidation of glyceraldehyde-3-phosphate, which occurs in the non-particulate fraction (cytoplasm). But no such energy-rich intermediates are found in the mithochondria.

2. *Conformational Coupling:* It is said that the electron carrier protein or a coupling protein factor undergoes conformational changes on account of the energy yielded in electron

transport. These changes result in the protein molecule reaching a higher energy level. This energy is transferred to ADP to form ATP with the simultaneous reversion of the protein to its lower energy level. An example of this type of coupling is the change occurring in the actomyosin formation accompanied by binding of ATP and its hydrolysis to ADP and phosphate. Not much evidence is available in favour of this hypothesis in the mitochondria.

3. *Chemiosmotic Coupling:* P. Mitchell, a British biochemist, postulated the chemiosmotic coupling hypothesis in 1961. According to this, an intact mitochondrial membrane is a prerequisite for oxidative phosphorylation. The membrane is normally impermeable to H^+ ions. The electron carriers of the respiratory chain help in actively pumping H^+ ions from the matrix across the inner mitochondrial membrane. An electrochemical gradient is thus generated, the outer side of the inner membrane rich in H^+ and the inner side rich in OH^- (due to loss of H^+). This helps in dehydrating ADP and phosphate and causes the formation of ATP from them. The detailed steps are shown in Fig. 13-2.

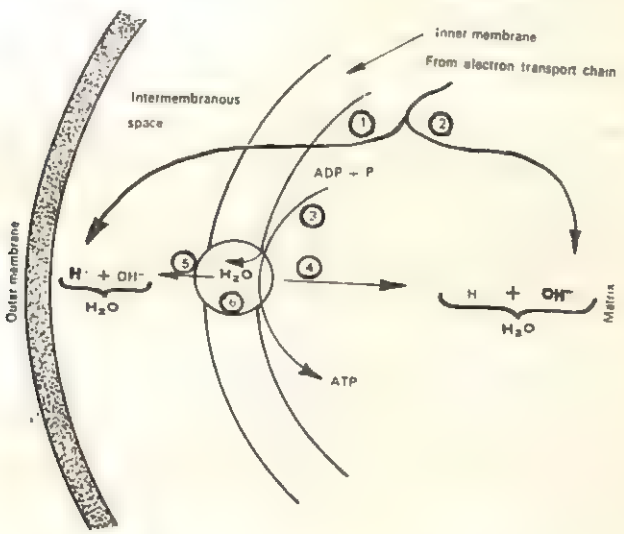


Fig. 13-2. Chemiosmotic coupling

1. During electron transport, the passage of a pair of electrons at each energy yielding zone generates two H^+ ions. The H^+ ions are pumped out by the inner membrane.

2. As a result, the pH of the intermembranous space is lowered and an electric potential is also generated across the inner membrane. The matrix accumulates OH^- ions.

3. The electromechanical gradient drives forward the reaction $ADP + P \longrightarrow ATP + H_2O$. The reaction occurs at the active site of the membrane (6) which contains the F_1ATPase complex.

4. The water molecule dissociates to H^+ (derived from ADP) and OH^- (derived from phosphate). The H^+ migrates into the matrix to combine with the OH^- there to form water.

5. Similarly, the OH^- migrates out to combine with H^+ which had been initially pumped out of the inner membrane. Equilibrium is thus restored.

This theory explains most of the features observed in oxidative phosphorylation. An intact mitochondrial membrane is needed for effective oxidative phosphorylation. The mitochondrial membrane is normally impermeable to H^+ ions. ATP formation is found to be associated with movement of H^+ ions and pH gradient across the membrane. 2,4-Dinitrophenol, by allowing free passage of H^+ ions across the membrane, prevents the building up of the eletrochemical gradient across the membrane and prevents formation of ATP. Hence the chemiosmotic coupling theory is generally accepted.

Action of Ionophores

Substances like *Valinomycin* and *Nigericin* have lipophilic character and allow penetration of the mitochondrial membrane by K^+ and H^+. Thus, they abolish the proton gradient and the pH gradient across the membrane and inhibit the chemiosmotic phosphorylation of ADP to ATP. These substances are called *ionophores*. Dinitrophenol has similar action.

Transport of Metabolites: The inner mitochondrial membrane is impermeable to a number of substances like NAD^+, $NADP^+$ and their reduced forms, AMP, acetyl-CoA and others. The mitochondrial pool of these substances is hence separate from the cytoplasmic pool. For certain other substances like ADP. ATP, pyruvate, citrate, succinate, α-ketoglutarate and malate, there are specific carrier or transport systems called 'carriers', 'translocases' or 'porters'. The ATP-ADP carrier is inhibited by atractyloside, a vegetable product.

For the transfer of these substances against gradient, the energy of electron transport may be required. This is particularly true of Ca^{++} transport. Mitochondria can transport Ca^{++} into the matrix against gradient. An equivalent amount of phosphate is also transported. The calcium transport occurs at the expense of ATP production. The accumulation of calcium and phosphate in the mitochondria is one of the steps in biological calcification.

Inhibitors of electron transfer

1. Cyanides: 1 to 3 m. moles (27-81 mg) is a lethal dose for the human. They act by tightly combining with cytochrome oxidase enzyme and prevent the final step in electron transfer.

2. *H_2S:* This acts in a similar manner and is equally toxic. It is a menace to oil-drilling workers, since large amounts of H_2S are present in oil-bearing strata.

3. *Antimycins:* They block the transfer of electrons from cytochrome b to c_1. They cannot be used as antibiotics, since they are equally toxic to human.

4. Piericidin A and Rotenone: They block electron transfer between NAD and ubiquinone. They block the transfer between the iron-sulfur proteins and ubiquinone. Transfer of electrons from flavoprotein system is not effected.

5. *Barbiturates:* They block NADH dehydrogenase.

Inhibitors of oxidative phosphorylation

1. *Oligomycins:* They combine with the stalk of the phosphorylating particles and prevent the conversion of ADP to ATP. They do not block electron transport.

2. *Atractyloside:* It blocks the permeability of the mitochondrial inner membrane to ATP by blocking transport.

3. *Bongkrekate:* It acts similar to atractyloside. Some of these poisons are used in research. They are also used as insecticides and pesticides. Some are present in plant and bacterial products and pose a potential danger. eg. Rotenone is present in the roots of some tropical plants and is used as a fish poison. Atractyloside is present in the rhizomes of certain plants. Antimycin, piericidin and oligomycin are products formed by different species of streptomyces.
Bongkrekate is a toxin produced by a Psuedomonas and can contaminate certain foods.

Uncoupling of oxidative phosphorylation

Normally the two processes of electron transport and phosphorylation of ADP occur smoothly side by side. For each pair of electrons transferred (or for each atom of oxygen utilized) three ATP are produced. A dissociation between the two processes will result in oxidation without generation of ATP and a lowering of the P/O ratio (ATP/oxygen).

2:4 Dinitrophenol: Being lipid soluble, it readily permeates the inner mitochondrial membrane and acts as a proton carrier and transfers protons from the cytosol to the matrix. The proton gradiant is therefore lost and ATP generation does not occur.

Valinomycin: This is produced by a type of Streptomyces. By its ionophore action, it transports K^+ from the cytosol into the matrix and H^+ from the matrix into the cytosol, thereby decreasing the proton gradient.

Enzymes and coenzymes of biological oxidation-reductions:

Oxidases: The hydrogen removed from the substrate is passed on directly to oxygen. They usually are copper containing enzymes.

$$AH_2 + \tfrac{1}{2}O_2 \xrightarrow{\text{oxidase}} A + H_2O.$$

eg: Cytochrome oxidase, phenolase (tyrosinase etc.), ascorbic acid oxidase, uricase and monoamine oxidase.

2. *Aerobic dehydrogenases:* They pass over the hydrogen either to oxygen or other acceptors like methylene blue. With oxygen, they form H_2O_2 and not H_2O. The enzymes are flavoprotein in nature. Some contain metallic ions also.

$$AH_2 + O_2 \longrightarrow H_2O_2 + A$$
$$AH_2 + MB \text{ (Methylene blue)} \longrightarrow M.B.\ H_2 + A$$

eg: D-and L-amino acid dehydrogenases, xanthine dehydrogenase and aldehyde dehydrogenase. They are also called oxidases. Glucose oxidase is another enzyme in this group.

3. *Anaerobic dehydrogenases:* They pass on the hydrogen to an intermediate acceptor – a coenzyme or another enzyme system.

1. $AH_2 + X \longrightarrow A + XH_2$

2. $XH_2 + B \longrightarrow X + BH_2$

$AH_2 + B \longrightarrow A + BH_2$

The hydrogen acceptor may be any of the coenzymes.

A. Nicotinamide coenzymes (NAD or NADP): Only one of the hydrogen atoms is taken up by the coenzymes, along with an electron from the second hydrogen atom. This leaves the second hydrogen atom in an ionic form.

$$NAD^+ + AH_2 \longrightarrow NADH + H^+ + A.$$

Alcohol dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase are two examples.

NAD is present to the extent of 0.4 to 2.0 mg. per gram of tissue, whereas NADP is present only from 0.01 to 0.1 mg. In liver they are about equally distributed. Most of NAD is present in the oxidized form (NAD^+) while NADP is in the reduced form ($NADPH + H^+$). Every cell has the ability to synthesize its own pyridine nucleotides.

NAD^+ is the coenzyme used in reactions leading to substrate oxidation and ATP generation.

$NADP^+$ is used in reactions where reducing power (as $NADPH + H^+$) is to be utilized for synthetic reactions.

The reduced forms of the coenzymes strongly absorb light at 340 mμ. They exhibit flourescence when activated by light at 340 mμ and emit light at 465 mμ. These absorption maxima are slightly altered if they are combined with their apoenzymes. Many of the NAD dependent enzymes contain zinc. In many instances, binding of the substrate can only occur after the coenzyme is first bound to the enzyme protein, which indicates stereo alteration in the enzyme configuration when combined with coenzyme (eg. lactic dehydrogenase does not combine with pyruvic acid unless it has combined earlier with NAD).

Pyridine nucleotide transhydrogenases: The enzymes are capable of transferring hydrogen between the two coenzymes - NAD and NADP.

$$NADPH + H^+ + NAD^+ \longleftrightarrow NADP^+ + NADH + H^+$$

B. Riboflavin coenzymes: FAD or FMN is the prosthetic group of the enzymes. It takes up hydrogen from the reduced NAD (the enzyme is named NADH dehydrogenase) or directly from substrates like succinic acid (eg. succinic dehydrogenase). In the case of acyl-CoA dehydrogenase, the flavoprotein also contains iron which is not a component of heme (non-heme iron or NHI). It is therefore a metalloflavoprotein. In such cases, another flavoprotein called the 'electron transferring flavoprotein or ETF' will act as carrier of electrons from the enzyme to the respiratory chain.

Nonheme iron. Iron-sulfur protein or Ferredoxins

These are associated with flavoproteins and cytochrome b. In addition to iron and sulfur, two or four atoms each – Fe_2 S_2 or Fe_4 S_4 – they also contain four thiol groups from four cysteine residues. They are components of NADH dehydrogenase complex and succinate dehydrogenase complex. They probably mediate in electron transfer to ubiquinone. '*Rhodanase*', an enzyme present in all cells, aids in the formation of the iron-sulfur centre. The enzyme also catalyzes interaction of thiosulfate and cyanide to form sulfite and thiocyanate, and thus helps in detoxication of cyanide.

Unlike the pyridine nucleotides, the flavine coenzymes are more firmly bound to the enzyme proteins. Flavoproteins exhibit characteristic absorption spectra with maxima at 280,380 and 450 mμ. In the reduced state, the absorption at 280 and 380 is decreased and that at 450 is altogether lost. In 50% reduced (or 50% oxidized) state, the absorption at 450mμ is one half and two additional bands appear with centres at 550 and 650mμ. FMN exhibits strong flourescence while FAD shows only slight fluorescence. Some flavoproteins contain one or more metal ions, a heme group, or an additional reducible organic group like ubiquinone. The molecular weights vary from 12,000 to 30,000.

C. Coenzyme Q: Also known as 'ubiquinone', it has a structure similar to vitamin K and exists in two forms – reduced (quinol) form and oxidized (quinone) form. It takes up hydrogen from the flavin coenzymes.

D. Cytochromes as coenzymes: They carry electrons only from the coenzyme-Q and pass them on to cytochrome oxidase (a_3), which, in turn, passes them on to molecular exygen. They are iron containing hemoproteins. The iron can undergo reversible oxidation and reduction to ferric and ferrous states. Cytochrome b also contains some non-heme iron (NHI).

The order of transport of electrons is from cytochrome b to c_1 to c to a to a_3.

Cytochromes are present in all aerobic organisms and their content is proportional to the respiratory activity of the tissue. Heart muscle and the flight muscle of insects and birds are richest. Next come the liver, kidney, brain and skeletal muscle. Skin and lung contain the smallest amounts.

They all exhibit characteristic absorption bands in the reduced state. They disappear or become diffuse and less intense on oxidation. Originally three cytochromes were described by Keilin – cytochromes a, b and c. They have absorption bands as shown in table below. Later, several other cytochromes were detected and were named b_1, b_2 etc., depending on their resemblance to one or other of the original cytochromes.

	alfa band	**beta band**	**gamma band**
Cytochrome a:	605 mμ	517 mμ	414 mμ
Cytochrome b:	563 mμ	530 mμ	430 mμ
Cytochrome c:	550 mμ	521 mμ	416 mμ

They are usually intimately associated with the mitochondrial protein-lipid complex. Of all the cytochromes, cytochrome-c can be extracted with ease by aqueous solvents. The others are more firmly bound to the insoluble particulate matter and are released only on treatment of mitochondria with detergents like deoxycholate or dodecylsulfate.

Cytochrome bc_1 complex: This is made up of eight subunits - cytochrome c_1 (m.w. 29,000), cytochrome b (m.w. 28,000), iron sulfur centre (m.w. 24,000) and five other subunits. The total molecular weight will be in the range of 200,000. Except cytochrome b which is encoded by mitochondrial DNA, the other subunits are all encoded by nuclear DNA.

Cytochrome c: The complete molecular structure and amino acid sequence of cytochrome c from several species of animals were established in detail. The horse heart cytochrome c is a globular structure containing 104 amino acid residues and a single heme attached to two cysteine residues. It does not react either with oxygen or carbon monoxide (i.e. it is not autooxidizable). It does not also react with cyanide. How it exactly mediates in electron transfer between cytochrome bc_1 complex and the aa_3 complex is not clear.

Cytochrome oxidase or Cytochrome aa_3 complex: This contains seven subunits. They are - cytochrome a_3 (m.w. 35,000), cytochrome a (m.w. 26,000) which contains copper, and five other subunits (with a total m.w. of 66,000). The aa_3 complex has a molecular weight of 400,000 and exists as a dimer (7+7 subunits). The system not only transfers electrons to oxygen, but also helps in pumping out protons into the cytosolic side of the mitochondrial membrane. Cytochrome aa_3 subunits are synthesized on mitochondrial and cytoplasmic ribosomes and are finally assembled to form the enzyme complex in the mitochondria. Carbon monoxide, cyanide and H_2S inhibit cytochrome oxidase.

Cytochrome b_5: It is a small molecule (m.w. 16,000) and occurs tightly bound to membranes of the endoplasmic reticulum.

It is a component of NADH-dependent Stearyl CoA desaturase system (which converts stearyl CoA to Oleil CoA). It is also involved in the biosynthesis of fatty acids (microsomal) in the step converting beta-ketoacyl CoA to beta-hydroxyacyl CoA and in the conversion of α, β - unsaturated acyl CoA to acyl CoA.

Cytochrome P_{450}: This can combine with carbonmonoxide in the reduced state to form a compound with absorption maximum at 450 nm. Hence its name. It occurs in most animal tissues, plants and microorganisms and catalyzes monoxygenation reactions.

$$RH + O_2 + NADH + H^+ \longrightarrow ROH + H_2O + NAD^+$$

It may accept the electrons directly from NADH or through an iron-sulfur protein. The omega carbon of fatty acid and the ring carbon of steroids are some of the examples of oxidation by this system.

4. Hydroperoxidases: They use hydrogen peroxide as substrate. They are flavoprotein enzymes and usually occur in organelles called peroxisomes. The enzymes peroxidase and catalase belong to this group.

$$AH_2 + H_2O_2 \xrightarrow[\text{Peroxidase}]{} A + 2H_2O$$

$$2H_2O_2 \xrightarrow[\text{Catalase}]{} 2H_2O + O_2$$

Peroxisomes: These are organelles containing a few flavoprotein oxidases which use molecular oxygen and produce H_2O_2, eg. D-amino acid oxidase. D-Amino acids are contained in many antibiotics. Peroxisomes also contain peroxidases which utilize the H_2O_2 formed for other oxidation reactions.

Superoxide dismutase and catalase:

During electron transport to oxygen in the respiratory chain, partial reduction products of oxygen like superoxide anion (O_2^-) and hydrogen peroxide are formed. They are highly toxic to the cell. They are removed enzymically as and when they are formed:

$$2O_2^- + 2H^+ \xrightarrow[\text{Superoxide dismutase}]{} H_2O_2 + O_2$$

$$2H_2O_2 \xrightarrow[\text{Catalase}]{} 2H_2O + O_2$$

The superoxide dismutase enzyme is associated with Mn^{++}, Ca^{++} or Zn^{++} depending on the tissue and the organelle where it occurs.

5. *Oxygenases:* They catalyze direct transfer of oxygen to a substrate molecule. They may be dioxygenases or monooxygenases.

Dioxygenases introduce two atoms of oxygen into the molecule to form two hydroxyl groups.

$$AH_2 + O_2 \longrightarrow A(OH)_2$$

Eg: Tryptophan pyrrolase (tryptophan 2, 3-dioxygenase). Monoxygenases or hydroxylases catalyze the insertion of a single oxygen atom. For the simultaneous utilization of the second oxygen atom in the oxygen molecule, they are coupled with other enzyme reactions:

$$AH + XH_2 + O_2 \longrightarrow AOH + H_2O + X$$

Eg: Phenylalanine hydroxylase (phenylalanine 4-monooxygenase).

In this reaction, tetrahydrobiopterin acts as a carrier of the reducing equivalents by its interconversion from the dihydro to tetrahydro form.

Molybdenum-containing hydroxylases:

Sulfate oxidase, xanthine oxidase and aldehyde oxidase are some of the molybdenum containing enzymes. The reaction is –

$$A + H_2O \longrightarrow + AOH + H^+ + 2e^-$$

The electrons enter the electron transport chain. The molybdenum is usually bound to a pterin moiety as molybdopterin. There is also a heme molecule in the case of sulfite oxidase. Xanthine and aldehyde oxidases contain flavo- proteins with iron-sulfur centres.

Bioluminescence:

In the firefly, the energy produced in biological oxidations is also utilized for the emmission of visual light. A heterocyclic phenol called LUCIFERIN and an enzyme LUCIFERASE are involved.

LH_2 ⟷ L

(Luciferin, reduced) (Luciferin, oxidized)

The return of the oxidized form to the ground state results in the emission of light, the color of which depends on the nature of the enzyme protein.

References:

Bray, H.G. and White K.: Kinetics and thermodynamics in Biochemistry 2nd ed., Acad. Pr., N.Y., 1966.

Chance, B. Estabrook, R.W. and Yonetani, T. (eds): Hemes and Hemoproteins, Acad. Pr., N.Y., 1966.

Edsall. J.T. and Wyman, J.: Biophysical Chemistry, vol. I., Acad. Pr., N.Y., 1958.

Extermann, R.C. (ed): Radioisotopes in Scientific Researh, vol. I., Acad. Pr., N.Y., 1958.

Florkin, M and Stotz., E.H.: Comparative Biochemistry, vol. 14, Biological Oxidations. Vol. 22, Bioenergetics, Amercian Elsevier, N.Y., 1966, 1967.

Kamen, M.D.: Radioactive Tracers in Biology: An Introduction to Tracer Methodology, 3rd. ed. Acad. Pr., N.Y., 1957.

Kaplan, N.C. and Kennedy, E.P.((eds): Current Aspects of Biochemical Energetics, Acad. Pr. N.Y., 1966.

Klotz, I.M.: Energy Changes in Biochemical Reactions, Acad. Pr., N.Y., 1967.

Krebs, H.A. and Kornberg, H.L.: Energy Transformations in Living Matter., Springer-Verlag. OHG, Berlin., 1957.

Lehninger, A.L.: The Mitochondrial Structure and Function, Benjamin., N.Y., 1964.

Singer, T.P. (Ed.): Biological Oxidations, Wiley., 1967.

Slater, E.C.: Flavoproteins, Amer, Elsevier., N.Y., 1966.

Umbriet, W.W., Burris, R.H. and Stanffer, J.F.: Manometric Techniques and Related Methods for the Study of Tissue Metabolism, 4th ed Burgess Pub. Co., Minneapolis., 1964.

Wagner., A.F. and Folkers, K.: Vitamins and Coezymes, Interscience., N.Y., 1964.

14

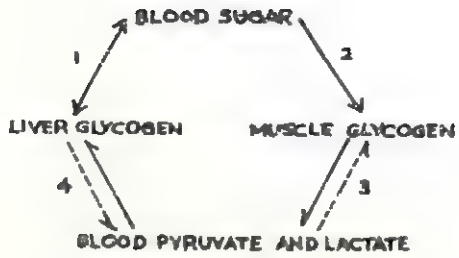
CARBOHYDRATE METABOLISM

GENERAL REVIEW

In whatever form carbohydrate has been ingested in food it is converted to the monosaccharide glucose and absorbed into the portal blood. Some of the monosaccharides absorbed in small amounts are the hexoses-fructose, galactose and mannose. They are carried by the portal blood to the liver. Liver has the ability to convert all other hexoses to glucose. Some of the glucose may enter the systemic circulation to supply the requirement of the other tissues. The surplus glucose is converted to glycogen and stored by the liver. This process is studied under the heading 'glycogenesis'. Glycogenesis occurs in other tissues also, notably muscle, as a means of storing surplus glucose made available from intestinal absorption. In a well-fed individual, about 40% of glucose is converted to fat, 'lipogenesis', and stored in the fat reservoir of the body, the adipose tissue.

The carbohydrate currency of the body is glucose and even in the fasting condition there is always a minimum amount of it, 60-90 mg. per 100 ml. in peripheral blood. During absorption from intestines, this may go up to about 110 to 140mg./100ml. and is rapidly taken up by all the tissues. In a few hours the blood sugar is restored to the fasting level. The fasting level is maintained constant by slow breakdown of glycogen from liver to produce glucose. The process is known as 'glycogenolysis'. Glycogen from muscle also breaks down, but not to glucose. During the breakdown of glycogen, the glucose ester, glucose-6-phosphate, is produced. This can be converted to glucose by an enzyme, glucose-6-phosphatase. The enzyme is present in liver but absent in muscle. Hence, in the muscle, it is not converted to glucose, but proceeds further in a process to be described as 'glycolysis' and forms pyruvic acid or lactic acid which comes out of the muscle into the blood stream, taken back to the liver and by a reversal of the process resynthesized to glucose or glycogen. The glucose (or glycogen) thus formed in liver is now available for maintenance of blood glucose level. The conversion of glycogen in the muscle to lactic acid can take place under anaerobic conditions (in the absence of O_2) and also yields energy which can be utilized by the muscle for contraction.

The cycle of events which connect up the liver glycogen through blood sugar to muscle glycogen and back through blood lactic acid to liver glycogen is known as the 'Cori's cycle'.



Note that reaction (1) is readily reversible; reaction (2) is not reversible; reaction (3) and (4) are reversible, but normally proceed only in one direction (shown by the continuous line).

Glucose and glycogen can be synthesized by the liver from substances which are derived from non-carbohydrate sources also, e.g. amino acids. This process is known as 'gluconeogenesis or glyconeogenesis'. During periods of prolonged starvation, when no carbohydrate is available from food, it is by gluconeogenesis that liver maintains a minimum fasting blood sugar level. This is necessary since the brain can utilize only glucose for its metabolism and cannot survive its lack even for short periods.

The maintenance of a blood sugar level within certain physiological limits under all conditions is known as 'homeostasis' of blood sugar level and is regulated by hormonal and nervous factors. (The reaction of the body to stimuli that tend to disturb some constituent in the body to restore the concentration to normal is termed *'homeostasis'* by Cannon).

To get the maximum available energy from glucose, it has to be oxidized completely to CO_2 and H_2O. The pyruvic acid (or lactic acid) produced by muscle or liver is oxidized to acetate which combines with a molecule of oxaloacetate to form citric acid. The citric acid runs through a cycle of events during which the acetate molecule is oxidized to CO_2 and H_2O with the production of large amount of energy and the oxaloacetate molecule is regenerated. This cycle of events is described under 'citric acid cycle'. Glucose is also metabolized through certain other pathways which are not so important for the energy production but are extremely important as synthetic pathways for a number of substances. 'Hexose monophosphate pathway' and the 'uronic acid pathway' are of this category .

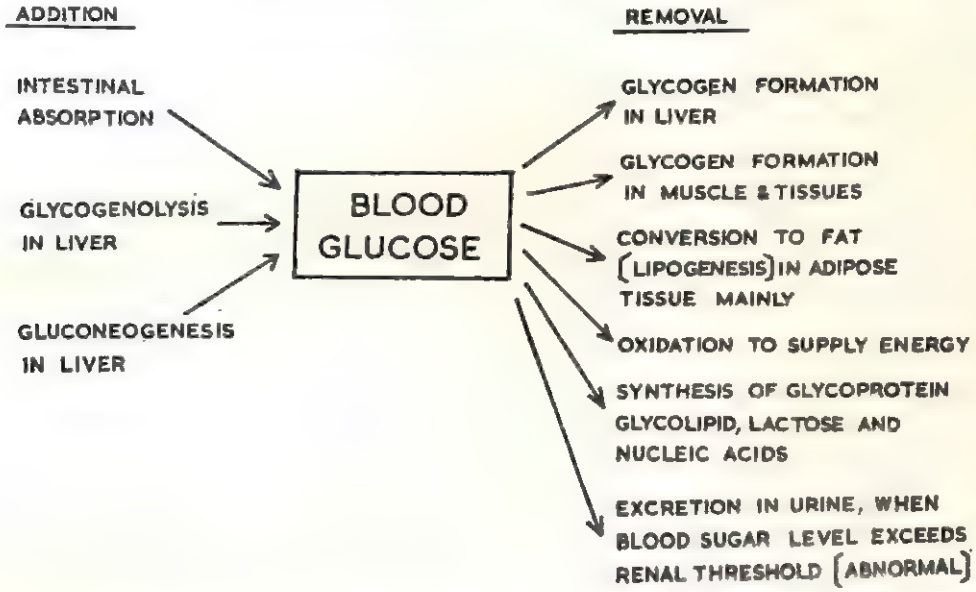
Regulation (homeostasis) of blood glucose level: The blood sugar level is maintained within physiological limits (60 to 90 mg/100 ml. in fasting state and 100-140 mg/100 ml. following ingestion of carbohydrate containing meal) by a balance between two sets of factors (i) factors adding glucose to the blood and (ii) factors that remove glucose from the blood.

These are summarized on page 247.

All these processes are under nervous and hormonal control.

A. In the post-absorptive state:

The fasting state is better described as the post-absorptive state which is the condition obtaining 12 to 14 hours after the last meal. There is no intestinal absorption at this time.



At the same time it is not prolonged starvation which itself leads to several abnormalities in metabolism. The condition of a subject between 8 a.m. to 10 a.m. if he had his dinner the previous evening about 8 p.m. and has taken nothing thereafter in the night or morning can be said to be the post-absorptive state.

Under these conditions, the only source of glucose to the blood is from liver glycogen. At rest the tissues of an adult man will be utilizing around 200 mg. glucose per minute by taking up the glucose from blood. This much will be added by the liver. The glycogen concentration of the liver averages 5%. Thus an adult liver weighing 1800 grams can store about 90 grams of glycogen which can supply at the rate of 200 mg/minute for only 7 or 8 hours. The skeletal muscle (about 28 kg. in an adult male) has a glycogen content of about 1% and can hold 280 grams of glycogen. This can indirectly supply glucose via blood lactate and liver glycogen for less than another 25 hours. But blood sugar levels are maintained fairly constant even during much longer periods of starvation. This is by the process of gluconeogenesis occurring in liver. Amino acids are mainly used for this conversion.

B. Post-prandial regulation:

The condition following ingestion of food is described as the postprandial state. There is active absorption of carbohydrate, protein and lipid from the intestines. Monosaccharides other than glucose are converted to glucose by the liver and in some instances by the intestinal

mucosa. Some glucose is allowed to enter the systemic circulation leading to a rise in the blood sugar level from the fasting level of 60-90 mg% to a postprandial level of 100-140 mg%. The liver will convert the rest of absorbed glucose to glycogen and store it.

The tissues also take up the glucose from circulation and convert it to glycogen for storage. In a well-nourished individual, where the glycogen stores of liver and tissue are fairly saturated, about 40% of the absorbed glucose is used for lipogenesis. Some amount of glucose is also utilized for the other synthetic processes (such as glycoprotein synthesis) and for production of energy by glycolysis and citric acid cycle.

When the load of glucose is very heavy, as may happen when glucose is administered intravenously, a final mechanism of regulation comes into operation. The renal tubule has a capacity to reabsorb about 250 to 350 mg glucose per minute. This is known as the 'tubular maximum for glucose reabsorption (abbreviated as Tm_g). When the blood sugar rises over 140 to 180 mg/100 ml, the amount of glucose filtered into the glomerular filtrate exceeds the limit and the extra amount is excreted in urine. The level of blood glucose above which the kidney starts excreting glucose in urine is referred to as the 'renal threshold for glucose'. The increased level of blood sugar is known as hyperglycemia and the excretion of glucose in urine as glycosuria. Both are abnormal conditions and do not occur in a normal individual when carbohydrate is abosrbed only by the intestinal tract, even if pure glucose were ingested, because the rate of absorption by the intestine is not that fast.

Transport of glucose into the cell

The extracellular fluid contains glucose at a much higher concentration than in the intracellular fluid. Yet the entry of glucose into the cell is not by passive diffusion. It is mediated by transport systems. Two types are described.

1. *Insulin-independent transport system:* The hepatocytes, erythrocytes and brain cells do not require insulin for entry of glucose into the cell. The transport protein is an oligomer (M.W. 200,000) containing four subunits of equal size. Glucose, galactose and fructose are transported into the cell. The transport is inhibited by phlorizin.

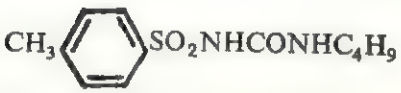
2. *Insulin-dependent transport system:* This functions in the muscle and adipose tissue cells. The binding of insulin to the receptors on the membrane enhances glucose transport into the cell by (i) causing migration of glucose transport proteins from the microsomal membrane, where they are synthesized, to the plasma membrane and (ii) by increasing the transport activity of the transport proteins.

In some of the lower organisms like the E. Coli, glucose transport into the cell requires expenditure of energy and phosphorylation of glucose to glucose-6-phosphate. But this is not the case with mammalian cells.

Role of hormones in the homeostasis of blood glucose levels.

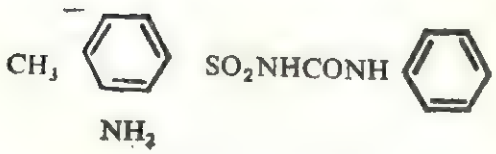
Insulin: This hormone of the β cells of the islets of Langerhans plays an important role. A rise in blood sugar stimulates β cells to secrete the homrone. The hormone enhances the uptake of glucose by tissues like muscle and adipose tissue, probably by enhancing the transport of the sugar across the cell membrane. It thus facilitates the storage of glucose as glycogen or lipid in this viscera. Conversely, in the liver, it suppresses glycogenolysis and gluconeogenesis — the two processes which supply glucose to the blood stream. These actions are mediated by its stimulating action on synthesis of some of the key enzymes concerned in glycogenesis and lipogenesis and suppressing the enzymes concerned in glycogenolysis and gluconeogenesis. The result is a lowering of the blood sugar concentration.

Oral Antidiabetic Drugs: Certain sulfonamide derivatives are found to stimulate increased insulin production by the beta cells of the pancreas. Tolbutamide, chlorpropamide and metahexamide belong to this group



(tolbutamide, Orinase)

(chlorpropamide, Diabines)

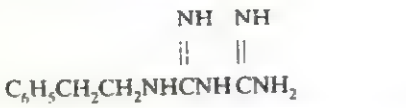


(metahexamide)

Since they act by increasing insulin production of a functioning pancreas, they are ineffective in alloxan diabetes and pancreatectomy diabetes of animals and in juvenile diabetes of man. They stimulate beta cells by a mechanism independent of glucose rise. They also accentuate the response of the beta cell to increases in blood glucose.

Phenethlbiguanide (Phenformin , DBI) and other biguanides can however act even in the juvenile diabetic and pancreatectomized and alloxan diabetic animal. This is becuase their action is independent of insulin. They cause uncoupling of oxidative phosphorylation in

tissues and enhance glycolysis and glucose uptake by the tissues, thus helping in removal of glucose from blood. They also decrease intestinal glucose absorption and hepatic gluconeogenesis. The overall result is a decrease in blood glucose levels. Phenethylbiguanide has the following structure.



Epinephrine (adrenaline): It is secreted by the adrenal medulla in response to hypoglycemia (lowered blood glucose levels). It is one of several hormones all of which antagonize the action of insulin. It stimulates glycogenolysis in the liver and muscle and leads to an increase in the blood glucose and lactic acid levels. The action is mainly on account of its activating the enzyme phosphorylase which is concerned in glycogenolysis.

Glucagon: This hormone is produced by the α-cells of the islets of Langerhans and, like epinephrine, its secretion also is stimulated by hypoglycemia. It stimulates glycogenolysis in liver by activating the liver phosphorylase. It also enhances gluconeogenesis in the liver. By these two mechanisms it increases blood sugar levels. It has however no action on muscle phosphorylase and hence does not increase the lactic acid levels of blood.

Adrenal cortical hormones: The hormones of the adrenal cortex which have a 'O' or a 'OH' in position 11 of the steroid ring are called the glucocorticoids. They influence glucose metabolism by increasing gluconeogenesis in the liver. They increase protein breakdown in tissues and make the amino acids available to liver for gluconeogenesis. They also decrease the uptake of glucose by tissues. The overall effect will be an increase in the blood sugar level.

Anterior pituitary hormones: Growth hormone, ACTH and other diabetogenic factors produced by the anterior pituitary tend to increase the blood glucose levels. The growth hormone increases lipolysis (breakdown of fat to fatty acids) and decreases glucose uptake by tissues. The action of ACTH is mainly through stimulation of adrenal cortex.

Thyroid hormones: They are said to enhance the rate of absorption of glucose from the intestine and also enhance glycogenolysis in the liver. Both factors tend to increase the blood sugar level. But, on the other hand, they enhance the metabolism of glucose and thus the blood sugar rise is only transient.

It may be seen that threre is only one hormone which has a blood sugar lowering effect while all the others have the effect of raising the blood sugar level. Anything which lowers insulin activity or raises the activity of the other hormones will produce hyperglycemia and glycosuria, a condition described as 'diabetes mellitus'.

Role of kidney in glucose metabolism: The kidney has the ability to form glucose from intermediates of glucose metabolism as well as from amino acids (gluconeogenesis). However, under normal conditions, this plays a minor role, if any, in blood sugar regulation.

Nervous regulation: The central nervous system does not play a major part in blood sugar regulation. Stimulation of the sympathetic causes hyperglycemia and glycosuria. The actions are similar to epinephrine injection.

Carbohydrate tolerance and its assessment: The ability of the organism to respond in maintaining blood sugar homeostasis in the face of a load of glucose absorbed from the intestine or administered parenterally is described as the carbohydrate tolerance or more specifically 'glucose tolerance' of the individual. Several forms of 'glucose tolerance tests' are devised for testing this ability.

Oral glucose tolerance test or G.T.T.: The test is conducted on a subject in the post-absorptive state. After obtaining a fasting sample of blood and urine, a dose of 50 or 100 grams of glucose dissolved in a tumblerful (300 ml) of water is given orally and samples of blood and urine are collected every half hour for a period of three hours. The blood samples are analyzed for glucose concentration and the urine samples are qualitatively tested for presence of glucose. The fasting sample of urine is also tested for acetone. The results are tabulated or presented in the form of a curve (See Fig. 14-1).

Normal G.T.T.: In a normal individual, the fasting blood sugar is 60 to 90 mg/100 ml. and rises to a peak of 100 to 140 mg/ml. in the course of one hour or one hour and a half. In the elderly individuals it may go upto about 170 mg/100 ml. Then it comes down and reaches slightly below the fasting level by 2½ hours due to the overaction of insulin stimulated by rise in blood glucose. It returns to fasting level by 3 hours (adrenaline action). All samples of urine will be negative for glucose and acetone.

Decreased glucose tolerance: In individuals suffering from diabetes mellitus (insulin lack or overactivity of the counter-insulin hormones) the fasting blood sugar will be higher than 90 mg/100 ml and will rise above 170 mg/100 ml following ingestion of glucose. The level will remain high for a longer time and may not return to fasting level even by 3 hours. The urine samples corresponding to blood sugar levels over 170 mg% may contain varying amounts of glucose, usually indicated as traces (below 0.5%), 1 + (0.5–1.0%), 2 + (1.0–2.0%) and 3 + (over 2.0%). Blood sugar levels over 170 or 180 mg% are refrerred to as hyperglycemia and the presence of sugar in urine as glycosuria. In severe cases of intolerance, urine may also contain acetone and other ketone bodies, a condition known as acetonuria or ketonuria.

Increased carbohydrate tolerance: This occurs in the opposite set of conditions – an overactivity of insulin or a decreased activity of pituitary, adrenal cortex or medulla and thyroid hormones. The fasting blood sugar will be 60 mg/100 ml. or lower and does not rise to more than 100 mg/100 ml. even after glucose administration . Towards the end of the test the blood sugar may fall to very low levels and produce symptoms of hypoglycemia characterized by profuse sweating, weakness, fainting and tremors. Urine will be negative for glucose and acetone bodies.

Other changes during glucose tolerance test:

Arterio-venous blood glucose levels: In the postabsorptive state, there is only a small difference between the glucose levels in the arterial and venous blood – 5 to 10 mg%. But following glucose administration, there is a well defined increase in the arterial blood glucose level over the venous, though both have increased over the resting level. This is on account of greater extraction of glucose by the tissues from the arterial blood during its passage through tissues. (For obtaining arterial blood samples, a superficial artery like the femoral is used. Capillary blood obtained by finger prick is almost similar to arterial blood in this respect and can be used).

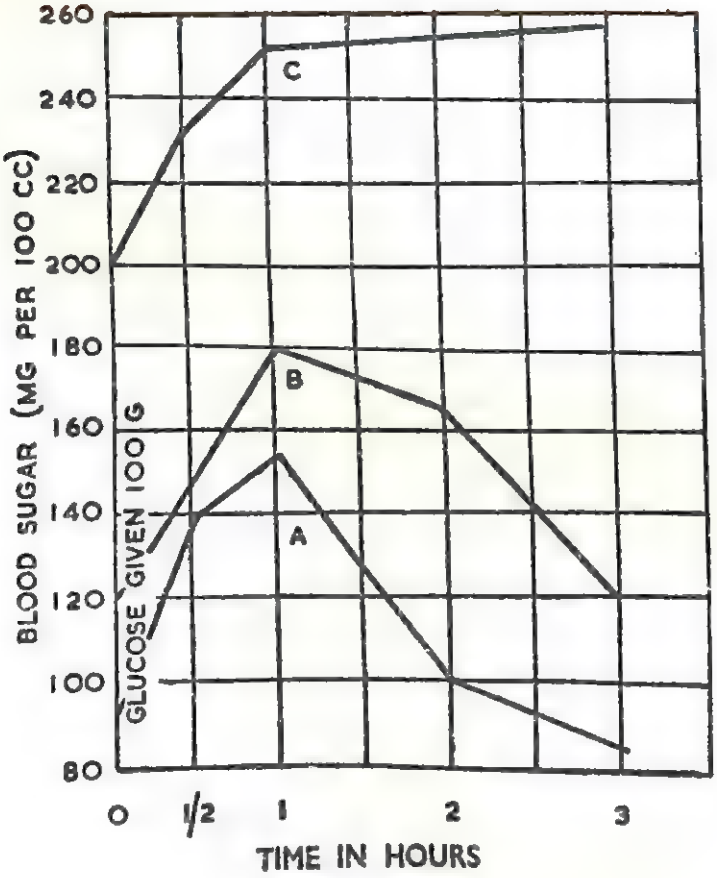


Fig.14-1.

A: Normal glucose tolerance.
B: Reduced glucose tolerance; mild diabetes mellitus.
C: Markedly reduced glucose tolerance; severe diabetes mellitus.

Serum inorganic phosphate: The uptake of glucose by the tissues and its utilization for glycogen synthesis or oxidation require its esterification with phosphate. The serum phosphate levels are therefore decreased during a glucose tolerance test.

Serum Potassium: Potassium moves into the cell along with glucose and results in a fall in serum potassium levels.

Respiratory quotient: This is about 0.82 in the postabsorptive state. Following glucose ingestion, due to increased carbohydrate metabolism, the R.Q. rises to a value nearer 1.0.

In diabetes mellitus, since carbohydrate metabolism is impaired, the above changes either do not occur or occur to a much less extent.

Factors altering carbohydrate tolerance:

1. *Quantity of glucose administered:* The peak level does not increase if the quantity of glucose ingested is over 50 grams since glucose is absorbed at a steady rate by the intestine. However, it may take a longer time to return to fasting level.

2. The rate of absorption may be low in conditions of malabsorption and high in hyperthyroidism. This may result in a low peak level or an early high peak.

3. *Diet preceeding the day of test:* To get a correct idea of the carbohydrate tolerance, the subject must be on a carbohydrate diet (at least 250 grams/day) for the preceding 3 days. Starvation or carbohydrate free diet will decrease the carbohydrate tolerance of the individual and cause hyperglycemia and glycosuria when a glucose load is given.

4. *Priming effect of a glucose dose one hour before the test:* If the test is done by administering 2 equal amounts of glucose, one following the other one or two hours, the peak reached with the second dose will be less than that with the first due to the increased responsiveness of the system. (Exton-Rose test for carbohydrate tolerance depends on this principle).

5. *Age:* Elderly people show a decrease in carbohydrate tolerance.

6. *Endocrines:* Insulin increases carbohydrate tolerance while all other hormones considered earlier tend to decrease its tolerance.

Intravenous glucose tolerance test: This eliminates the variable factor involved in the rate of intestinal absorption in different individuals. A 20% solution of glucose is infused in half an hour to supply 0.5 grams/kg body weight. In a normal person the concentration of blood glucose does not rise to more than 250 mg% at the end of infusion and falls below the fasting level in 2 hours.

Chemical processes involved in glucose metabolism:

The different pathways of metabolism of glucose are summarized schematically in Fig 14-2.

GLYCOGENESIS

The synthesis of glycogen from glucose can occur in most tissues in the body. Quantitatively muscle and liver are the most important sites of synthesis. Liver is the only viscera which can synthesize glycogen from monosaccharides other than glucose.

1. *Phosphorylation of glucose:* For glucose to take part in any metabolic process – be it synthesis to glycogen or breakdown by glycolsis or HMP pathway or uronic acid pathway – it is necessary for it to undergo phosphorylation to form glucose-6-phosphate. This is mediated by an enzyme, hexokinase, (which can act on other hexoses like galactose and fructose also) or by a specific enzyme, glucokinase. Both enzymes are present in the higher mammals.

$$\text{Glucose} + \text{ATP} \xrightarrow[Mg^{++}]{\text{Glucokinase or hexokinase}} \text{Glucose-6-phosphate} + \text{ADP}$$

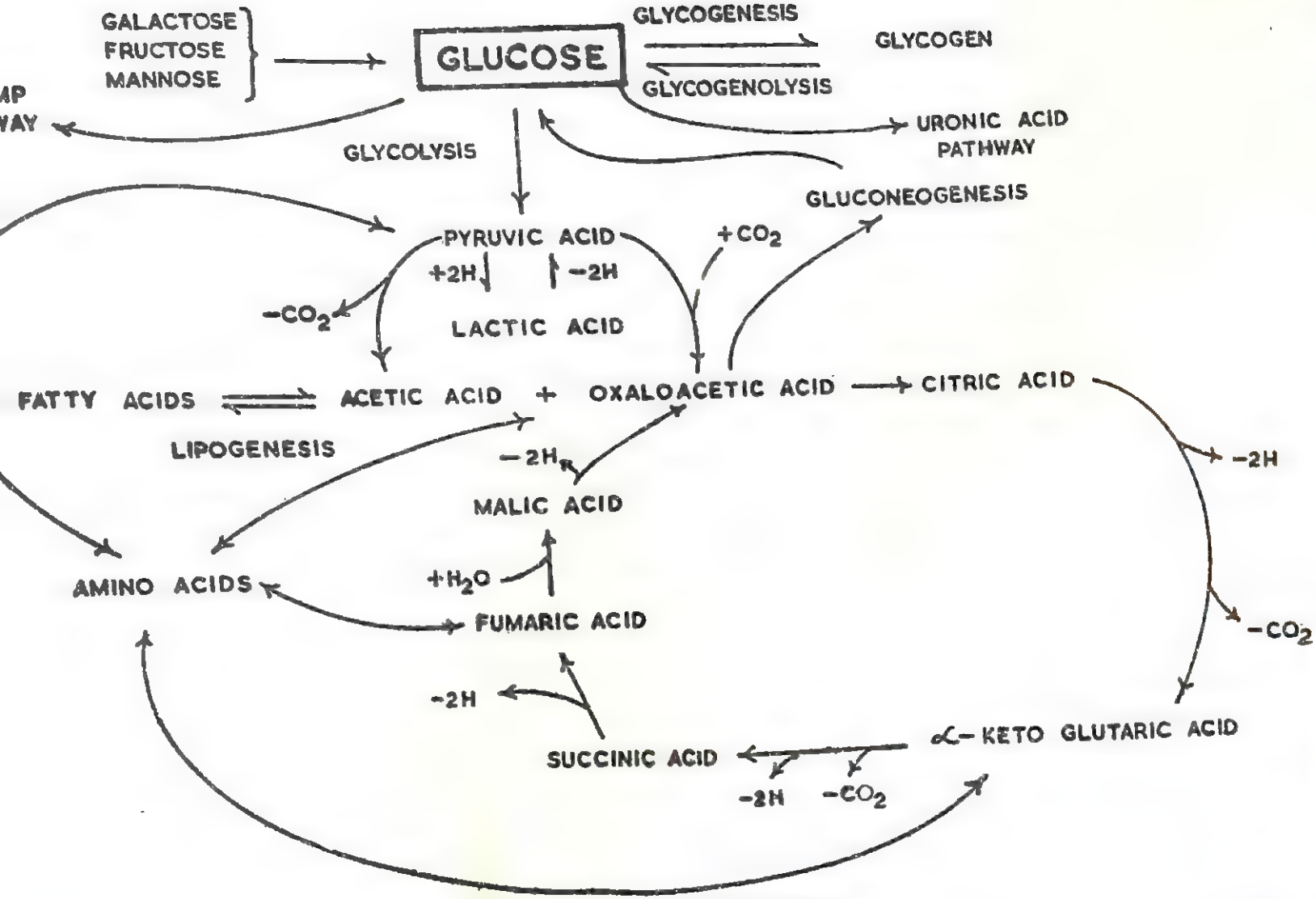


Fig. 14-2. Summary of Glucose Metabolism

Hexokinase is widely distributed and is the enzyme normally used by most cells. It can act on many hexoses and their derivatives including D-fructose, D-mannose and D-glucosamine. It has a greater affinity for aldohexoses than ketohexoses. It is a regulatory enzyme and is inhibited by its own product, glucose-6-phosphate.

Glucokinase, on the other hand, acts only on D-glucose. It has a much higher K_m for D-glucose and thus requires a high concentration of glucose to become fully active. It is not inhibited by its product, glucose-6-phosphate.

In liver, glucokinase predominates. It is absent in muscle. It therefore comes into activity in liver when blood glucose concentrations are increased. It is deficient in the livers of diabetic patients. Mg^{++} or Mn^{++} are required for the activity of the two enzymes. The ATP combines with the divalent cation ($MgATP^{=}$ or $MnATP^{=}$) and acts as the substrate for the enzyme.

Fetal liver contains only hexokinase. Glucokinase appears at birth and reaches adult levels in two weeks. Glucokinase levels are decreased in fasting and in diabetes mellitus. Refeeding or insulin injections will restore the levels to normal. Epinephrine and glucagon inhibit this restoration of glucokinase to normal on refeeding. Adrenal cortical hormones aid in restoration of the levels on refeeding.

Formation of glucose-6-phosphate is a 'locking mechanism' to keep the glucose within the cell, since it is not permeable to cell membrane. Glucose is readily permeable.

$$\text{2. Glucose-6-P} \xrightarrow[Mg^{++}]{\text{Phosphoglucomutase}} \text{Glucose-1-P}$$

This is a reversible reaction brought about by the mutase enzyme.

Phosphoglucomutase: The enzyme contains a serine residue which is essential for its activity. Mg^{++} and glucose 1, 6-diphosphate are also required. It is possible that the enzyme, phosphorylated at the serine residue, transfers its phosphate to glucose phosphate (at 1 or 6 position) to form a diphosphate and then the phosphate from the other carbon (6 or 1 position) is removed by combining with the serine residue of the enzyme:

$$\text{EP} + \text{G–6–P} \longrightarrow \text{E} + \text{G 1, 6–P}$$
$$\longrightarrow \text{EP} + \text{G – 1-P}$$

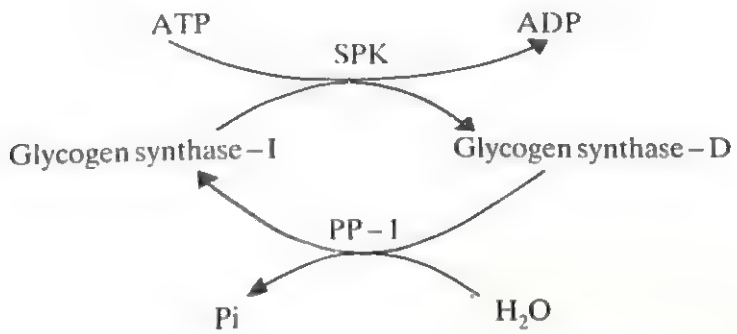
$$\text{3. Glucose-1-P} + \text{UTP} \xrightarrow{\text{Pyrophosphorylase}} \text{UDP–Glucose} + \text{PP}$$

The gluscose-1-P interacts with uridine triphosphate (UTP) and combines with it to form uridine diphosphate glucose (UDP-glucose) after releasing a molecule of pyrophosphate.

$$\text{4. UDP-Glucose} + \text{Glycogen (primer)} \xrightarrow[\text{(Transglucosylase)}]{\text{Glycogen synthase}} \text{UDP} + \text{Glycogen (richer by one molecule of glucose).}$$

The glycogen synthetase is an enzyme also called UDPG-glycogen-transglucosylase. It requires a preformed glycogen molecule (a primer) for its action. The glucose molecule from UDP-glucose is transferred and added to the end-glucose of one of the branches of glycogen by 1-4 glycosidic linkage. This reaction is driven forward when excess of glucose-6-phosphate is present. As a result of this reaction, a preexisting chain of a branch of glycogen is lengthened.

Glycogen synthase (synthetase) exists in two forms - the I and D forms - the I form independent of glucose-6-phosphate concentration and considered to be the active form; the D form depends on the concertration of glucose-6-phosphate and is considered to be the inactive form. The I form is converted to the D form on phosphorylation. Conversely, the D form can be converted to the I form by dephosphorylation. Phosphorylation and dephosphorylation are brought about by enzymes called 'synthase phosphorylase kinase' (SPK) and 'phosphoprotein phosphatase' (PP-1) respectively. The process is under hormonal control.



ATP phosphorylates SPKb (inactive) to form SPKa (active form). The conversion is mediated by a cyclic AMP dependent protein kinase. The activity of SPK enzymes are dependent on Ca^{++} ions. Calmodulin -Ca^{++} combination or troponin -Ca^{++} combination can further activate the enzyme. These are released from the sarcoplasmic reticulum during muscle contraction. Thus, glycogenolysis is intimately connected with muscle contraction.

Phosphoprotein phosphatase (PP-1) removes phosphate not only from phosphorylase but also from SPK. AMP and glucose-1-phosphate inhibit the enzyme allosterically. Glucose-6-phosphate, glucose and glycogen stimulate the enzyme. Cyclic AMP inhibits the enzyme.

Epinephrine acts on all these enzymes by its stimulation of adenyl cyclase and production of cyclic AMP. Glucagon has no action on the muscle enzyme.

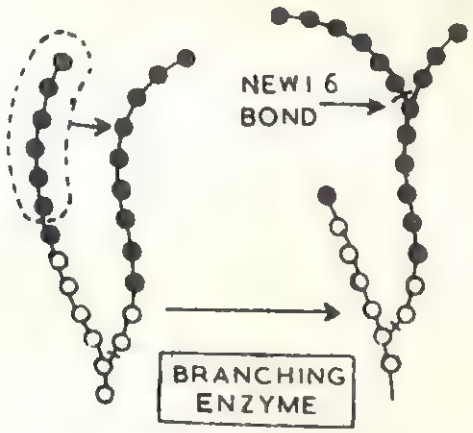
Glycogen synthase is a tetramer (M.W. 350,000) with identical subunits and is tightly bound to glycogen granules.

While phosphorylation activates phosphorylase, it inactivates glycogen synthase. Thus the SPK, by phosphorylating the two enzymes – glycogen synthase and phosphorylase – promotes

glycogenolysis. The enzyme *Phosphorprotein phosphatase (PP-I),* by removing phosphate from the two enzymes, promotes glycogen synthesis.

In the resting muscle, synthase I predominates. In the contracting muscle, synthase D predominates. Epinephrine inhibits glycogen synthesis through production of cyclic AMP which acts through the cascade system of reactions.

5. Branching enzyme (or amylo-1, 4- 1, 6-transglucosidase) will act on the chain after it has been sufficiently lengthened by the synthetase. In the growing glycoside chain with glucose units linked by 1, 4-glycosidic linkage, this enzyme will remove chain fragments of 6 or 7 glucose units and reattach the fragments, at spaces of 8 to 12 glucose units apart on the original chain by α -l, 6-linkage to form branches. (See figure).



By the combined action of the last two enzymes the size of the molecule of glycogen grows.

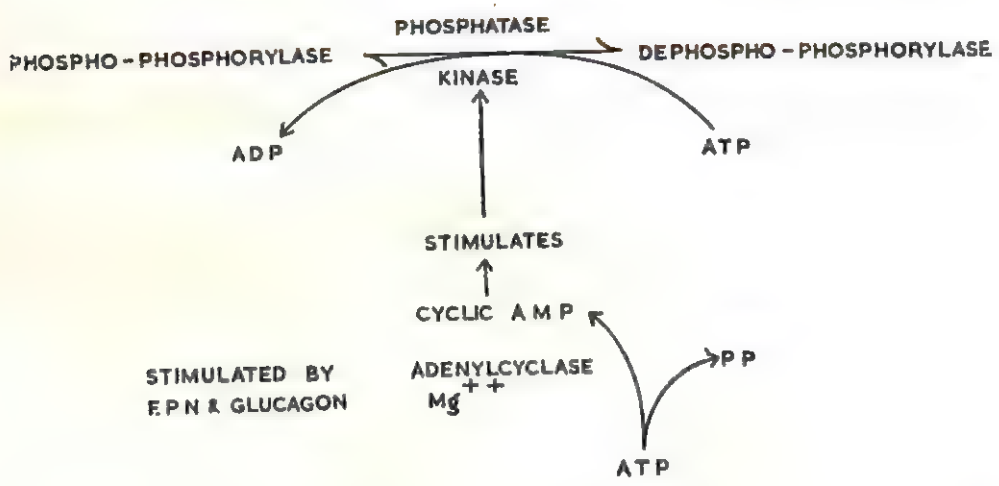
GLYCOGENOLYSIS

The breakdown of glycogen to glucose follows the reverse set of chemical reactions, but is mediated by different enzymes.

1. Glycogen + Phosphate $\xrightarrow{\text{Phosphorylase}}$ Glucose-1-P + Glycogen (one glucose molecule less).

The phosphorylase is a key enzyme in glycogenolysis (just like hexokinase in glucose metabolism in general). Liver phosphorylase differs in some respects from muscle phosphorylase.

Liver phosphorylase: The active form of liver phosphorylase contains phosphate esterified to a serine moiety in the enzyme. The enzyme becomes inactive on loss of the phosphate by hydrolysis by an inactivating enzyme. It can be reactivated by addition of phosphate derived from ATP. The active enzyme containing phosphate is called phospho-phosphorylase.

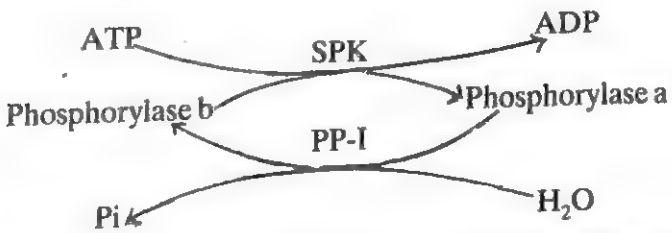


The reactivation is brought about by a 'kinase' enzyme which requires cyclic AMP (3', 5' -adenylic acid). The cyclic AMP can be formed from ATP by the action of the enzyme adenyl cyclase. Epinephrine and glucagon stimulate adenyl cyclase and thus provide more of cyclic AMP which in turn stimulates through the 'kinase' the production of phospho-phosphorylase (active phosphorylase). Thus the two hormones stimulate glycogenolysis in the liver.

Muscle Phosphorylase: This also exists in an active (a) form and an inactive (b) form.

Phosphorylase (M.W. 194, 800) is a dimer containing two identical subunits and occurs tightly bound to glycogen granules; 'a' is the phosphorylated form and 'b' the dephosphorylated form. Phosphorylation requires ATP and occurs at the -OH groups of serine in each subunit of the 'b' form to produce the 'a' form.

Phosphorylation is brought about by the same 'synthase phosphorylase kinase -SPK' and dephosphorylation by the same 'phosphorprotein phosphatase - PP-I' which were considered under glycogen synthesis.



The catalytic site of phosphorylase contains pyridoxal phosphate combined with the eta-amino group of a lysine residue. The phosphate group of pyridoxal phosphate acts as a proton acceptor during catalysis. The enzyme loses all activity if pyridoxal phosphate is removed. The b form of the enzyme requires cyclic AMP for its activity. Glucose and UDP-glucose inhibit the enzyme.

Resting muscle contains phosphorylase b mainly. This can produce the minimal amount of glycolysis required in the resting muscle. During muscular activity, the b form is converted to the a form which is more active. This conversion is brought about by SPK enzyme which itself exists in an active and an inactive form (SPK-a and SPK-b), and is under hormonal control, as already considered.

2. Phosphorylase attacks successively the glucose units at the end of each chain till only 4 glucose units remain in the chain.If glycogen is subjected to the action of phosphorylase alone, it will result in the formation of a glycogen molecule with each branch having only 4 glucose units - what is called the *"limit dextrin"*.

A second enzyme now comes into action. It is called *"oligo* (α - 1,4 $\rightarrow$ α - 1,4) - *glucan transferase"* and transfers the three glucose units containing the 1,4-glycosidic linkage to a new chain and unites them by α -1,4 glycosidic linkage, thus extending the chain length by 3 glucose molecules. Phosphorylase can now act on these added glucose units.

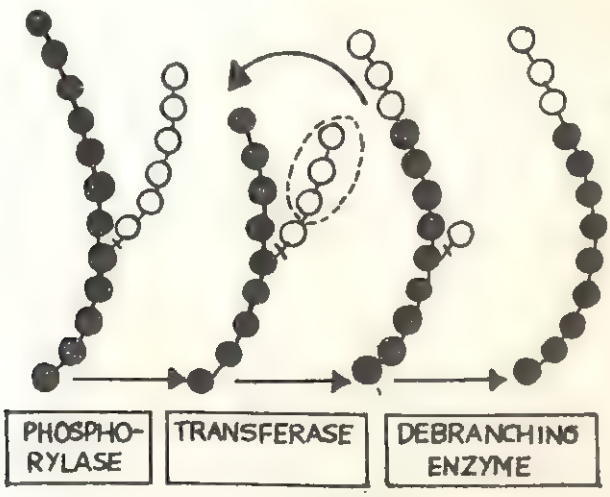
3. The last remaining glucose unit of the first chain united by 1-6 linkage is now removed by amylo-1, 6-glycosidase (debranching enzyme) releasing a molecule of glucose (and not glucose-1-phosphate). (See figure).

$$\text{Glycogen} \xrightarrow[\text{(at the branching point, 1, 6 linkage)}]{\text{Debranching enzyme}} \text{Glycogen + Glucose (1 glucose molecule less)}$$

4. $$\text{Glucose-I-P} \xrightarrow[Mg^{++}]{\text{Phosphoglucomutase}} \text{Glucose-6-P}$$

This is the reverse of similar reaction in glycogen synthesis.

5. $$\text{Glucose-6-P} \xrightarrow{\text{Glucose-6-phosphatase}} \text{Glucose + P}$$



This enzyme required for the final production of glucose from glycogen is present only in liver (and kidney) but is absent in muscle. Hence liver only can contribute glucose to the blood.

Glycogenesis and glycogenolysis are summarized schematically in Fig. 14-3.

Glycogen Storage Disease

There is abnormal deposition of glycogen in the tissues (especially liver and muscle) due to inherited deficiency of one or the other of the enzymes concerned in the synthesis or breakdown of glycogen. The different types are tabulated in Table 14-1 and shown in Fig. 14-4.

Table 14-1

Glycogen Storage Disease

Type and Common Name	Enzyme Deficient	Tissues in which glycogen accumulates etc.
Type I (von Gierke's Disease)	Glucose-6-phosphatase	Lliver, kidney. Blood sugar levels low and not increased by epinephrine/glucagon.
Type II (Pompe's Disease)	Lysosomal α-1, 4- and –1, 6-glucosidases	Lysosomes
Type III (Limit Dextrinosis)	Debranching enzyme	The glycogen stored resembles limit dextrins.
Type IV (Amylopectinosis)	Branching enzyme	Glycogen has very few branches.
Type V (McArdle's Syndrome)	Muscle phosphorylase	Muscle. Poor tolerance to exercise. Blood lactate does not rise after exercise or epinephrine injection.
Type VI (Hers' Disease)	Liver phosphorylase	Liver

Types VII, VIII, IX & X are also described due to defects in enzymes like phosphofructokinase, glycogen synthase, phosphorylase kinase and protein kinase.

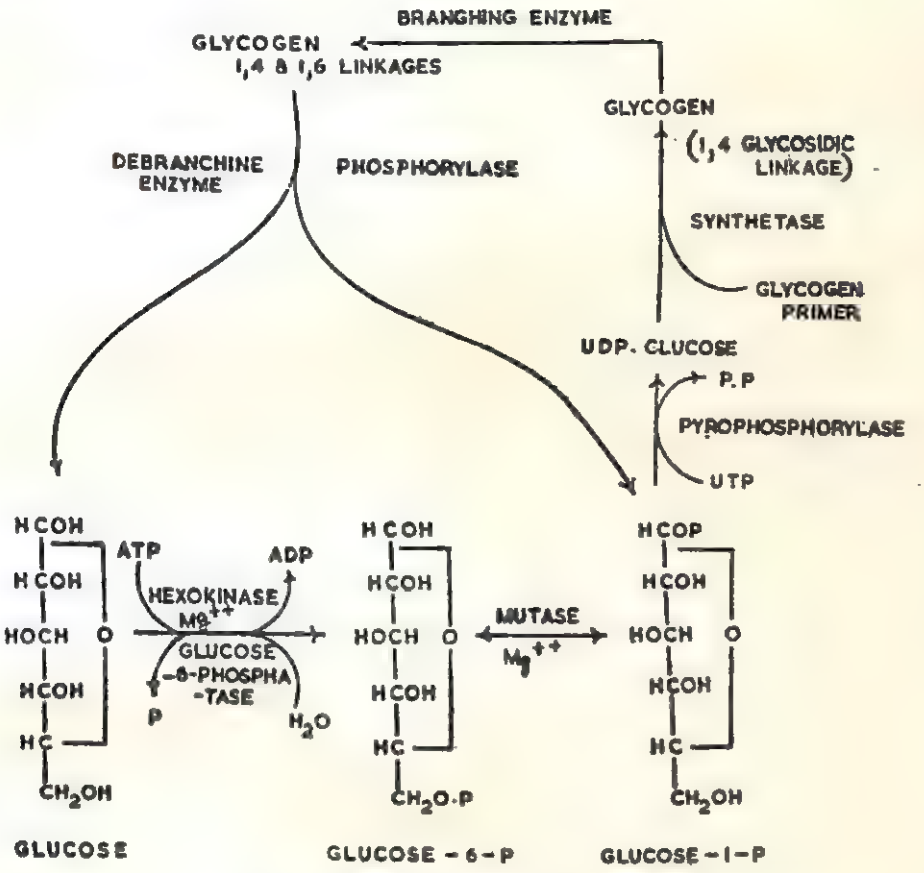


Fig. 14-3. Glycogenesis and Glycogenolysis

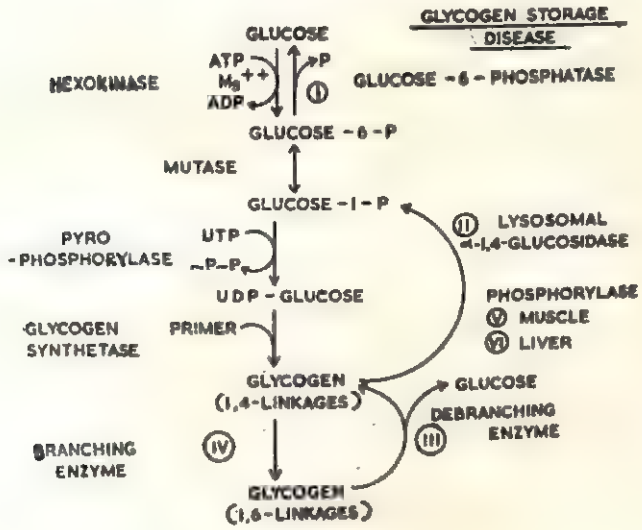


Fig. 14-4

INTER-CONVERSION OF HEXOSES

Galactose and fructose can be readily converted to glucose and then to glycogen (see Fig. 14-5).

Galactose: In a reaction catalyzed by galactokinase enzyme, galactose can be converted to galactose-1-phosphate. ATP is broken down to ADP in the process.

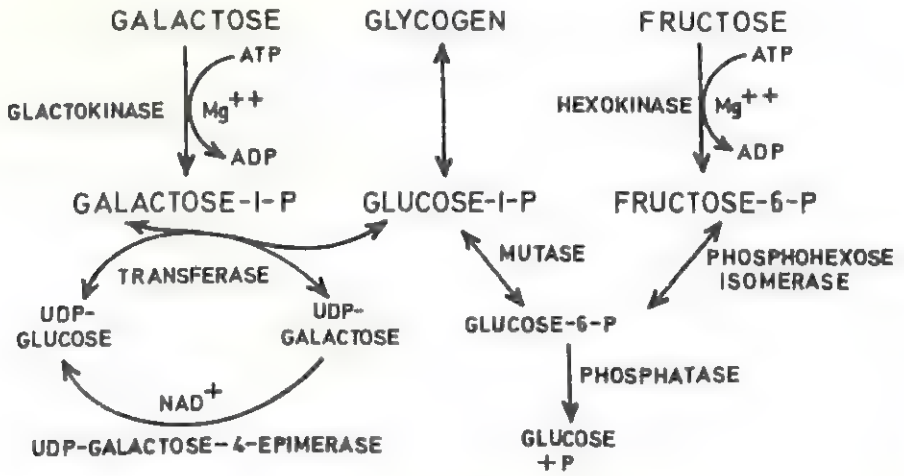


Fig. 14-5. Inter-Conversion of Hexoses

The galactose-1-phosphate interacts with a molecule of UDP-glucose (see under glycogen synthesis) to form glucose-1-phosphate and UDP-galactose. The reaction requires an enzyme 'galactose-1-phosphate uridyl transferase'. The UDP-galactose formed is converted to UDP-glucose by an 'epimerase' requiring NAD^+ as coenzyme. From UDP-glucose, glucose-1-phosphate can be liberated, to be converted to glucose-6-phosphate and glucose or the UDP-glucose may take part in glycogen synthesis.

Galactosemia: This is an inborn error of metabolism and occurs in newborn infants. They have an inability to convert galactose to glucose due to deficiency of the enzyme galactose-1-phosphate uridyl transferase. Galactose accumulates in blood and tissues and is excreted in urine. Aminoaciduria and ketonuria may also occur. Hepatomegaly, mental retardation and cataract of the lens are also seen. If milk is withheld from the infants' diet and substituted by lactose-free food, the condition is alleviated.

In the absence of normal metabolism of galactose, some of it is converted to its alcohol from — *galactitol* — by reduction. This is mainly responsible for cataract production.

Fructose: Hexokinase converts fructose to fructose-6-phosphate which is converted by an isomerase to glucose-6-phosphate which can be converted to glucose or enter glycogen synthesis.

Alternately, an enzyme, fructokinase, phosphorylates the first carbon of fructose to form fructose-I-phosphate. This is split by aldolase-B to dihydroxyacetone phosphate and glyceraldehyde. They can be metabolized further in the glycolytic pathway.

Hereditary deficiency of fructokinase causes *'Essential Fructosuria'*.

Absence of the enzyme 'Aldolase-B' gives rise to a form of hereditary fructose intolerance. The conversion of fructose to glucose can occur only in the liver and intestines. While fructose can be utilized with ease in the liver and adipose tissue, brain and muscle can utlilize it only after its conversion to glucose.

Seminal plasma, placenta and amniotic fluid contain free fructose. It is synthesized by these viscera from glucose.

GLYCOLYSIS — ANAEROBIC PATHWAY OR EMBDEN-MEYERHOF PATHWAY OF GLUCOSE METABOLISM

The breakdown of glucose or glycogen in muscle and other tissues is similar to the fermentation of glucose brought about by yeast organisms. In either case pyruvic acid is formed first. Under anaerobic conditions, the pyruvic acid is converted to lactic acid in mammalian tissues whereas in fermentation it is converted to ethyl alcohol.

$$CH_3CO\,COOH \xrightarrow{\text{Tissues}} CH_3CHOH\,COOH$$

lactic acid

$$CH_3CO\,COOH \xrightarrow[\text{yeast}]{} CH_3CH_2OH$$

ethyl alcohol

The reactions involved in the conversion of glucose (or glycogen) to pyruvic acid or lactic acid are known as 'glycolysis' or the Embden-Meyerhof pathway. This pathway of glucose metabolism is also called anaerobic pathway because it can take place in the absence of oxygen. There is only one oxidative step (dehydrogenation) in the conversion of glucose to pyruvic acid in which the coenzyme NAD^+ is reduced to $NADH + H^+$. If oxygen is available, the reduced coenzyme is reoxidized to NAD^+ through the respiratory chain. The pyruvic acid itself gets converted to acetate and is utilized in synthetic reactions or is oxidized in the citric acid cycle.

Under anaerobic conditions, the reduced NADH + H^+ is reconverted to the oxidized form of NAD^+ by passing on the hydrogen to the end product, pyruvic acid, which is reduced to lactic acid. Hence formation of lactic acid is only a side reaction in glycolysis to regenerate the coenzyme for further use in glycolysis. Otherwise the steps in glycolysis remain the same whether oxygen is available or not.

The chemical reactions involved are outlined in Fig. 14-6.

1. The first step in glycolysis is the 'hexokinase' reaction whereby glucose is converted to glucose-6-phosphate. ATP provides the phosphate as well as the energy for the synthetic reaction, and is converted to ADP. Magnesium ions are also required as activators. The reaction can be brought about by the enzyme 'glucokinase'.

2. The glucose-6-phosphate is converted to its isomer, fructose-6-phosphate by the action of 'phosphohexose isomerase' in the presence of magnesium ions.

3. The fructose-6-phosphate is again acted upon by a kinase enzyme, 'phosphofructokinase', to form fructose-I, 6-diphosphate. One more molecule of ATP is broken down to ADP and magnesium ions are required as activators.

Phosphofructokinase of the liver is an allosteric enzyme and is activated by fructose-2, 6-diphosphate, which is synthesized when glucose supply is plentiful. Glucagon, by decreasing the levels of fructose-2, 6-diphosphate, causes an increase in gluconeogenesis while inhibiting glycolysis.

4. The hexose diphosphate is now split up into two triose phosphate molecules — glyceraldehyde-3-phosphate and dihydroxyacetone phosphate by the action of 'aldolase'.

5. The triose phosphates are readily interconvertible by the action of 'phosphotriose isomerase'. Since subsequent steps utilize only glyceraldehyde-3-phosphate, it can be considered that two molecules of that substance are formed as a result of reactions 4 and 5.

6. Glyceraldehyde-3-phosphate now undergoes dehydrogenation and phosphorylation to form 1, 3-diphosphoglyceric acid by the action of the enzyme 'glyceraldehyde-3-phosphate dehydrogenase'. The enzyme is made up of four identical polypeptides, each containing an –SH group. However, only one of the –SH groups takes part in the reaction (hence the enzyme is represented as ESH) to form an enzyme-substrate complex with glyceraldehyde phosphate at the aldehyde group (first carbon). A thiohemiacetal is formed. Two hydrogen atoms from the complex are now taken up by NAD^+ to form NADH + H^+. The final step involves phosphorolysis whereby the enzyme is released and phosphate is added to the carbonyl group at position I. The sulfur bond of thiohemiacetal is energy-rich and this energy is retained in the phosphate bond which replaces it. These reactions are detailed in fig. 14-7.

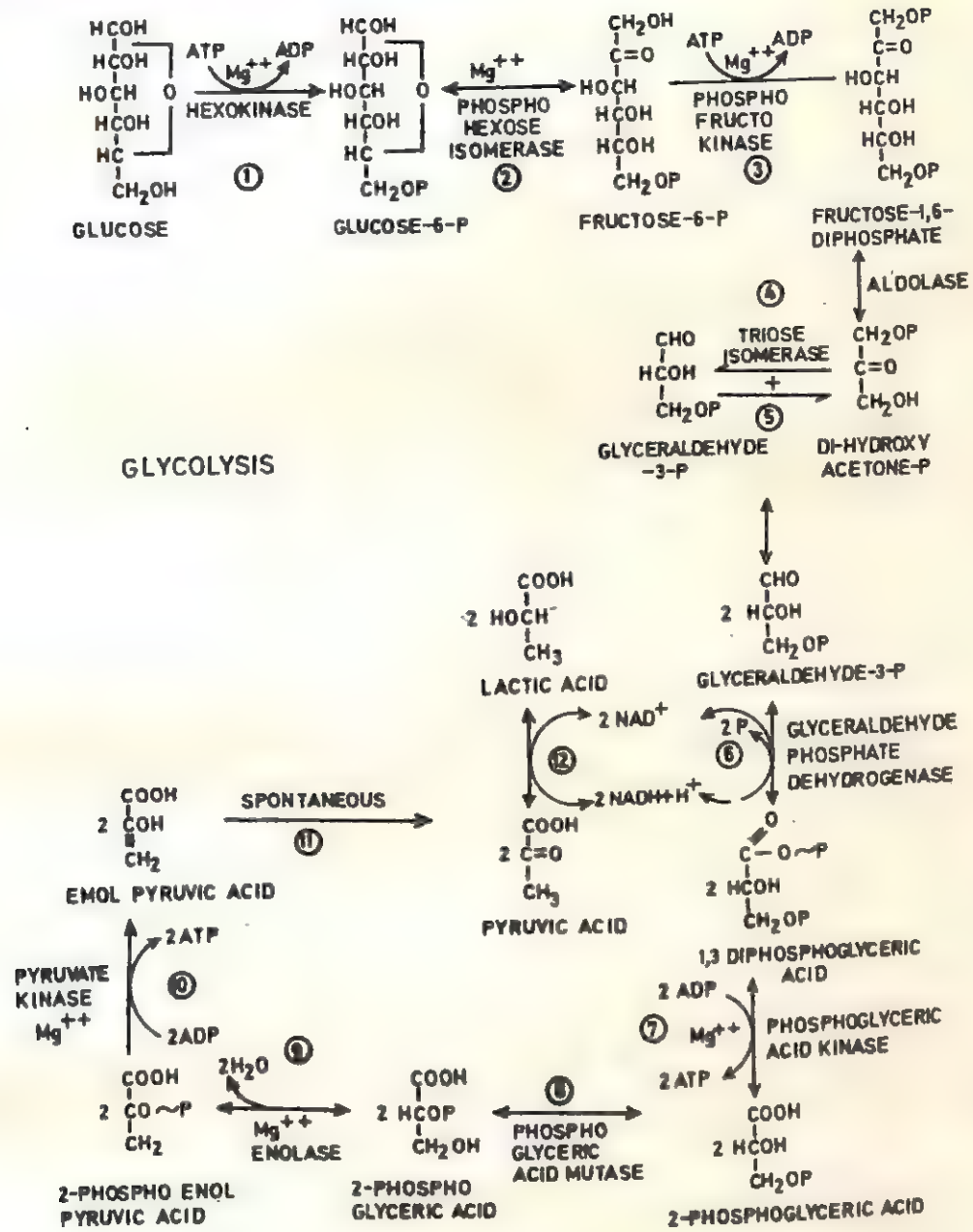


Fig. 14-6. Glycolysis (E-M Pathway)

Being an –SH enzyme, its activity is enhanced by cysteine and glutathione and inhibited by iodoacetate. Arsenate does not inhibit the enzyme but will replace phosphate in the first carbon to form 1-arseno-3-Phosphoglycerate. This is later hydrolyzed to give 3-phosphoglycerate, but the production of ATP in the next step (step 7) by transfer of

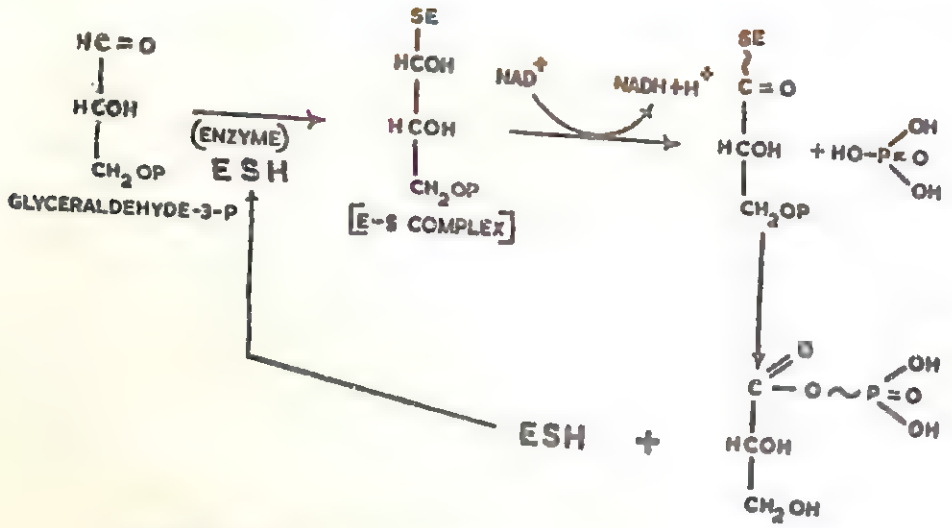


Fig. 14-7. Mode of action of Glyceraldehyde-3-Phosphate Dehydrogenase

energy-rich phosphate from the first carbon cannot occur. This is an example of uncoupling of oxidation from phosphorylation.

7. 'Phosphoglycerate kinase' in the presence of Mg^{++} will now transfer the energy-rich phosphate from the C-1 of 1, 3-diphosphoglyceric acid to ADP to form an ATP, leaving 3-phosphoglyceric acid.

8. The 3-phosphoglyceric acid is converted to 2-phosphoglyceric acid by the action of 'phosphoglyceric acid mutase'.

Phosphoglyceromutase requires for its activity the presence of 2, 3-diphosphoglycerate.

9. The enzyme 'enolase' will now remove a molecule of water from 2-phosphoglyceric acid to form 2-phosphoenolpyruvic acid. This results in a redistribution of energy to make the enolic phosphate bond an energy rich one. Enolase is inhibited by fluoride.

10. The energy-rich phosphate of phosphoenolpyruvate is transferred to ADP to form ATP and enolpyruvic acid by the enzyme 'pyruvate kinase'. Magnesium ions are required as activators. Pyruvate kinase also is a regulatory enzyme.

ATP and alanine inhibit the enzyme. Fructose-1, 6-diphosphate is a positive effector and drives the reaction forward. Glucagon inhibits the enzyme through cyclic AMP.

11. Enolpyruvate, being unstable, is spontaneously converted to pyruvic acid (keto form). The reaction is non-enzymic.

12. If conditions are aerobic, pyruvic acid will be further converted to active acetate and gets oxidized in citric acid cycle or used for lipogenesis. If conditions are anaerobic, it is converted to lactic acid by the action of 'lactic dehydrogenase' by taking up hydrogen from reduced NAD which is formed in reaction 6.

This will enable glycolysis to proceed under anaerobic conditions by providing a continuous supply of NAD (oxidized form) for the action of glyceraldehyde-3-phosphate dehydrogenase in reaction 6.

Lactate dehydrogenase: The M_4 isoenzyme is predominant in skeletal muscle. It has a low K_m and a high V_{max} for pyruvate. Hence it rapidly converts the pyruvate formed in the skeletal muscle to lactate.

The H_4 isoenzyme which is predominantly present in the heart muscle has, on the other hand, a high K_m and a low V_{max} for pyruvate and is inhibited by high levels of pyruvate. Hence, pyruvate is not normally converted to lactate in the cardiac muscle. It is oxidized in the citric acid cycle.

All enzymes required for glycolysis are cytoplasmic (extra-mitochondrial). Except the reactions (1) hexokinase, (3) phosphofructokinase and (10) pyruvate kinase the former two requiring ATP and the last one producing ATP, all other reactions are reversible.

Energy production in glycolysis: This can be assessed by the net gain in ATP.

			ATP used	*ATP gained*
Rean.	(1)	Hexokinase	1	—
"	(3)	phosphofructokinase	1	—
"	(6)	by reoxidation of the 2 ($NADH + H^+$) in the respiratory chain (P/O ration = 3)		2 x 3 = 6
"	(7)	phosphoglycerate kinase		2
"	(10)	pyruvate kinase		2
			–2	+10

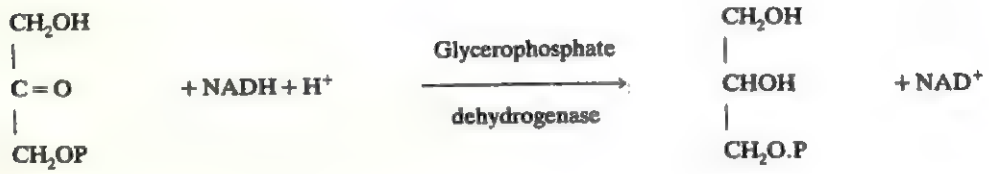
There is thus a net gain of 8 ATP, if the glycolysis stops with pyruvic acid and the $NADH + H^+$ of reaction (6) is reoxidized in the respiratory chain. If, on the other hand, it is under anaerobic conditions, and the coenzyme is reoxidized by passing the hydrogen to pyruvic acid to form lactic acid, then only 2 ATP (8–6) are gained.

The production of ATP following reation (6) is an example of oxidative phosphorylation whereas the production of ATP in reactions (7) and (10) are examples of substrate phosphorylation.

Glycerophosphate shuttle:

The $NADH + H^+$ produced in the glyceraldehyde-3-phosphate dehydrogenase reaction is in the cytosol and is not permeable to the mitochondria. In order to enter the mitochondria it follows a pathway called the "glycerophosphate shuttle".

The dihydroxyacetone phosphate formed in the aldolase reaction is reduced to alfa-glycerophosphate by glycerophosphate dehydrogenase.

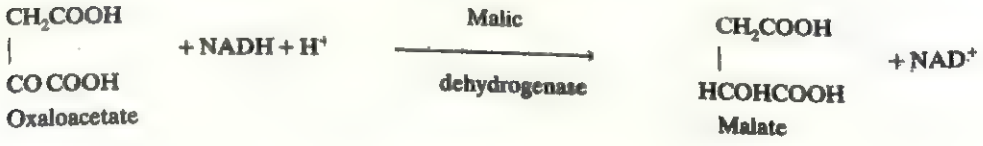


The glycerol phosphate is permeable to the mitochondria where the reverse reaction is brought about by the mitochondrial enzyme. But the mitochondrial enzyme uses flavo-protein as coenzyme.

The reoxidation of the flavin coenzyme in the electron transport chain generates only 2 ATP and not three. Hence the actual number of ATP produced in glycolysis where pyruvate is the end-product is 4+2+2–2=6 ATP. Where lactic acid is the end product, the ATP production is only 2+2–2=2 ATP per each glucose molecule.

Malate-aspartate Shuttle:

This is similar to the glycerophosphate shuttle. The NADH + H^+ formed in the mitochondria is also impermeable and cannot come out into the cytosol. This is circumvented by reducing oxaloacetate to malate in the mitochondria. The malate can readily pass out into the cytosol and again part with its hydrogens to NAD^+ to form NADH + H^+.



The oxaloacetate cannot re-enter the mitochondria, but can enter after transamination and formation of aspartate. If malate-aspartate shuttle is used for the transport of NADH + H^+ into mitochondria, the full complement of 8 ATP can be obtained from glycolysis, where the end product is pyruvic acid.

Oxygen debt: Under anaerobic conditions (say during strenuous muscular exercise) large amounts of lactic acid accumulate. These are later reconverted to pyruvic acid, and further oxidized by regeneration of the NADH + H^+ in the respiratory chain using oxygen. Thus, there is an increased demand for oxygen for some time following strenuous exercise. The increased demand of O_2 is to clear off the oxygen debt entered into by the tissues during the anaerobic phase.

Blood lactic acid: In resting condition, blood lactic acid is 5–20 mg/100 ml (0.5-2.0 mM/litre). During mild exercise like walking, it may rise to 5 mM/litre. During strenuous exercise, it may go up to 20 mM/litre. But the rise is only transient and is restored back to normal with rest.

Lactic acidosis: In some conditions, there is a persistent rise in blood lactate levels over 2 mM/litre even at rest. The pH is lowered and the condition is called lactic acidosis. This occurs in conditions like anoxemia, severe anemia, lack or deficiency of enzymes of gluconeogenesis, leukemia, shock, anesthesia, diabetes mellitus, alcohol toxicity and phenformin intoxication.

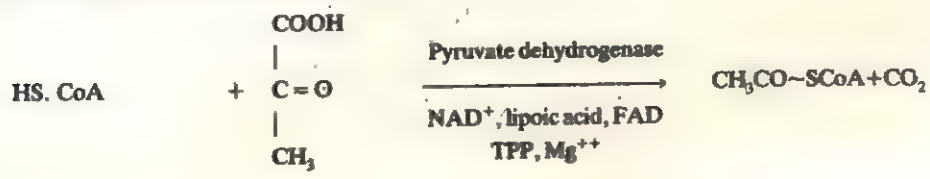
Inhibition of glycolysis: Apart from iodoacetate which inhibits the glyceraldehyde-3-phosphate dehydrogenase, sodium fluoride inhibits the enzyme enolase. In the estimation of blood sugar levels in the laboratory, it is found that low values are obtained in stored samples due to glycolysis by erythrocytes and leukocytes. This can be prevented by addition of sodium fluoride to the freshly collected sample of blood.

Mammalian erythrocytes metabolize glucose by glycolysis only since the enzymes required for the oxidation of pyruvate are not present in them.

CITRIC ACID CYCLE (KREB'S CYCLE)

This is the aerobic pathway and follows the anaerobic path from the pyruvic acid stage. The pathway starts conventionally with the formation of citrate by the condensation of oxaloacetate with acetate, both of which can be formed from pyruvate. Thus the conversion of pyruvate to acetate is an obligatory step in the utilization of carbohydrate by this pathway. This itself is an oxidative step and occupies a key position in glucose metabolism. One of the carbons of pyruvate is removed as CO_2 in the conversion. The remaining two carbons are removed as CO_2 in the citric acid cycle. There is a simultaneous oxidation of hydrogen through coenzymes in both. All the enzymes involved in the aerobic pathway are located mainly in the mitochondria along with the respiratory chain. A few of them occur in cytoplasm also.

Conversion of pyruvate to acetate:



This reaction is called 'oxidative decarboxylation' and is brought about by the enzyme 'pyruvate dehydrogenase'. During the reaction a coenzyme-A molecule is attached to the product to form acetyl coenzyme-A, otherwise known as active acetate. The reaction takes place in several steps as shown in Fig. 14-8.

The pyruvate dehydrogenase is an enzyme complex located in the mitochondrial matrix and consists of three enzymes and five coenzymes.

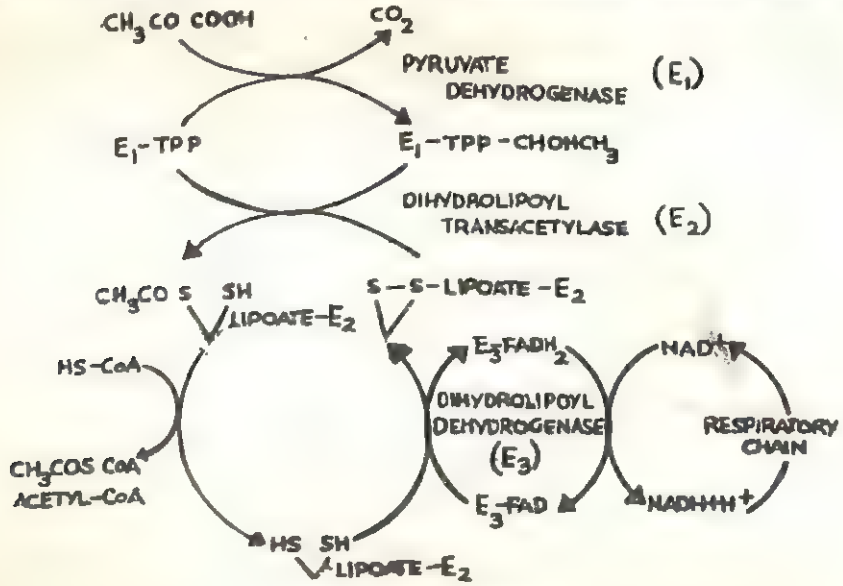
The enzymes are:
1. Pyruvate dehydrogenase
2. Lipoate acyltransferase and
3. Lipoamide dehydrogenase.

The coenzymes are: 1) TPP, 2) lipoic acid, 3) FAD, 4) Coenzyme A and 5) NAD^+.

Closely associated with this complex are also two other enzymes — pyruvate dehydrogenase kinase and pyruvate dehydrogenase phosphatase.

Pyruvate dehydrogenase kinase will inactivate the enzyme, pyruvate dehydrogenase, by phosphorylating it. It requires ATP and Mg^{++} and is inactivated by high levels of ATP. Pyruvate dehydrogenase phosphatase removes the phosphate from the inactive enzyme and activates it. This also requires Mg^{++}. It is also stimulated by Ca^{++}. These two enzymes help to regulate the pyruvate dehydrogenase activity.

The pyruvic dehydrogenase complex is capable of bringing about the several stages in the conversion of pyruvate to acetyl-CoA and in the reoxidation of reduced lipoic acid.



The reduced lipoic acid is reoxidized to S-S form and the two hydrogen atoms are taken up by FAD to be later oxidized through the respiratory chain to a molecule of water.

The thioester bond in acetyl coenzyme-A is energy rich and can readily make the energy available for synthetic reactions. Hence it is called an active acetate molecule. In addition, the oxidation of the 2 hydrogens through the coenzymes and respiratory chain will yield 3 molecules of ATP for each pyruvate converted to acetate. Active acetate can be formed not only from pyruvate but also by oxidation of fatty acids and from other sources. This is considered further under lipid metabolism.

The active acetate combines with a molecule of oxaloacetate to form a molecule of citrate and this can be taken to be the starting point of Kreb's cycle. The reactions involved in the cycle are outlined in Fig. 14-9.

The pyruvate dehydrogenase complex is inhibited by NADH + H^+, acetyl CoA and cyclic AMP.

Citric acid cycle reactions:

1. The cycle starts with the condensation of a molecule of acetyl-CoA with oxaloacetate to form citric acid. The condensation is brought about by 'citrate synthetase'. Citrinyl-CoA is first formed and later hydrolyzed to citric acid and coenzyme-A.

2. A molecule of water is removed from citrate to form cisaconitic acid.

3. The molecule of water is added again, but the H^+ and OH^- are added at different sites to form a molecule of isocitric acid. Reactions 2 and 3 are brought about by the same enzyme 'aconitase' which requires iron ions as activator.

4 and 5. Isocitric acid now undergoes dehydrogenation brought about by 'isocitrate dehydrogenase' to form oxalosuccinic acid. Mammalian tissues contain two distinct enzymes — one enzyme requiring $NADP^+$ and Mn^{++} and another requiring NAD^+ and Mg^{++}. The dehydrogenation step produces oxalosuccinate. But this is never released from the enzyme. While still in the E-S complex, it is further decarboxylated and alfaketoglutaric acid is released. Isocitrate dehydrogenase is a regulatory enzyme in citric acid cycle. It is an allosteric enzyme inhibited by ATP and activated by ADP.

6. The alfa-ketoglutarate now undergoes oxidative decarboxylation by the action of an enzyme complex 'alfa-ketoglutarate dehydrogenase'. It requires thiamine pyrophosphate (TPP), lipoic acid, NAD^+, FAD, coenzyme-A and magnesium ions. The reaction is similar to the oxidative decarboxylation of pyruvate to form acetyl-CoA. The product in this case is succinyl-CoA. The bond linking CoA to succinic acid is energy rich.

The reaction is inhibited by arsenite.

7. 'Succinate thiokinase' converts succinyl-CoA to succinic acid. The energy released by hydrolysis of the –CoA bond is utilized for the conversion of a molecule of guanosine-di-phosphate or inosine-di-phosphate (GDP or IDP) to the respective triphosphates, GTP or ITP. These can later interact with ADP to form ATP. This is the only step in citric acid cycle resulting in substrate phosphorylation.

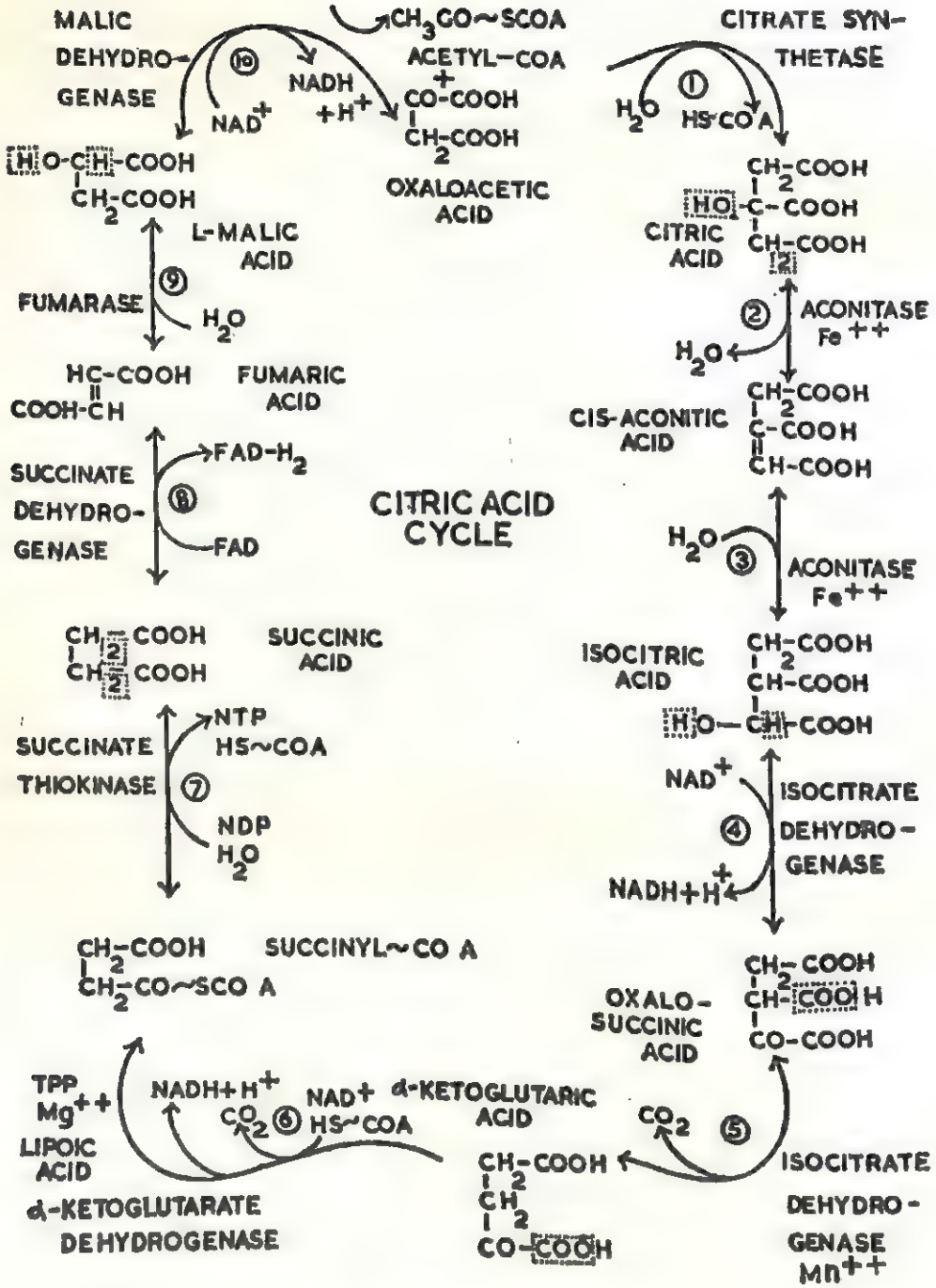


Fig. 14-9. Reactions of citric acid cycle.

8. Succinic acid loses two atoms of hydrogen to form fumaric acid. The enzyme concerned is 'succinic acid dehydrogenase'. The hydrogen is transferred direct to FAD contained in the flavoprotein of the enzyme without the intervention of NAD^+

Succinic dehydrogenase is an enzyme consisting of two subunits. Each subunit contains nonheme iron (FeS_2). The enzyme is a constitutent of the inner mitochondrial membrane: (the other enzymes of the citric acid cycle are present in the matrix).

The enzyme is inhibited by malonate, which, by its structural resemblance to succinate, causes competitive inhibition.

9. Fumaric acid takes up a molecule of water by the action of 'fumarase' to form malic acid.

10. Malic acid now undergoes dehydrogenation to form finally oxaloacetic acid, which is one of the two components that formed citric acid to start the cycle. The other component, acetate, has been oxidized in the cycle.

'Malic dehydrogenase' is the enzyme required for this reaction, and NAD^+ acts as coenzyme.

All reactions in the cycle except (1) and (6) are freely reversible. In reaction (1) citrinyl coenzyme-A is formed first. Coenzyme-A is then hydrolytically removed leaving citric acid. This reaction is inhibited by fluoroacetate which forms with oxaloacetate the compound fluorocitrate. This is not metabolized further. Hence the cycle cannot continue. Citrate synthetase is the rate-limiting step in the cycle. In addition to fluoroacetate, $NADH + H^+$ and ATP also inhibit the enzyme. ADP, inorganic phosphate and NAD^+ stimulate the enzyme. There are four dehydrogenation reactions (4), (6), (8) and (10) resulting in the formation of 4 molecules of water and two are utilized in reactions (7) and (9) leaving a surplus of two. Two molecules of CO_2 are liberated in reactions (5) and (6).

Thus the overall reaction is:

$$CH_3COOH + 2O_2 \longrightarrow 2CO_2 + 2H_2O.$$

Anaplerosis: Theoretically, if acetyl-CoA is supplied, the cycle can run indefinitely since oxaloacetate is reformed at each turn of the cycle. However, the intermediates of the citric acid cycle like oxaloacetate and alfa-ketoglutarate may also be utilized for the synthesis of amino acids like aspartic and glutamic and from them by transamination, several other non-essential amino acids. They have then to be replenished from other sources. The process of replenishment is called anaplerosis. Oxaloacetate can be synthesized from pyruvate by the addition of CO_2.

$$CH_3CO\,COOH + CO_2 + ATP \xrightarrow[\text{Biotin. } Mn^{++}]{\text{Pyruvate carboxylase}} \underset{\displaystyle CO\,COOH}{\overset{\displaystyle CH_2\,COOH}{|}} + ADP + P$$

Once oxaloacetate is formed, the other intermediates can be formed from it.

Conversely intermediates of citric acid cycle like oxaloacetate and alfa-ketoglutarate may be derived from the corresponding amino acids — aspartic and glutamic.

Thus citric acid cycle serves not only for catabolism but also to generate intermediates for anabolism. Hence, citric acid cycle is said to be *"amphibolic"*.

Energy production: No ATP is used in the reactions. The gain in ATP is by reoxidation of the reduced coenymes formed in reactions (4) (6) (8) and (10) and one substrate phosphorylation occurring in reaction (7).

			Coenzyme oxidized	*No. of ATP formed*
Rean	(4)	Isocitrate dehydrogenase	$NADH+H^+$	3
"	(6)	α -ketoglutarate dehydrogenase	$NADH+H^+$	3
"	(7)	Substrate phosphorylation by succinyl thiokinase	Succinyl CoA ↓ Succinic acid	1
"	(8)	Succinic dehydrogenase	$FAD.H_2$	2
"	(10)	Malic dehydrogenase	$NADH+H^+$	3
				12

12 ATP are produced for each acetate molecule oxidized in the cycle. In reaction (7) the substrate phosphorylation produces guanosine triphosphate (GTP) or inosine triphosphate (ITP) which is similar to ATP. They are interconvertible. In reaction (8), the coenzyme reduced is FAD and the step involving NAD is dispensed with. Hence the energy release is only 2 ATP, instead of the usual 3.

Total energy output from glucose:

		No. of ATP
During glycolysis from each glucose molecule		8
Conversion of 2 pyruvate to 2 acetate molecules	—2× 3=	6
Oxidation of 2 acetate in citric acid cycle	—2×12=	24
		38

In actual practice, only 36 ATP are obtained since in glycolysis there is a deficit of 2 ATP due to the glycerophosphate shuttle.

Glycolysis followed by citric acid cycle is the main pathway of glucose metabolism for the production of energy. Between the two, citric acid cycle is more productive of energy. Glycolysis alone may supply the immediate requirements of energy in conditions of strenuous muscular exercise where enough oxygen is not available for completing oxidation in citric acid cycle. Since only 2 molecules of ATP are produced per molecule of glucose under strictly anaerobic conditions leading to formation of lactic acid, a large amount of glucose has to be converted to lactic acid. When this exceeds certain limits, fatigue of the muscle occurs.

When aerobic conditions are restored, excess of lactic acid produced is reconverted to pyruvic acid and then oxidized in citric acid cycle to regenerate the ATP and creatine phosphate stores in muscle and to restore the coenzymes to their oxidized forms. Any surplus pyruvic acid is resynthesized to glycogen and stored.

GLUCONEOGENESIS

When glucose is not available in adequate amounts from dietary sources, its requirements are met by synthesis from non-carbohydrate sources. A continuous supply of glucose is necessary for the metabolism of brain, erythrocytes and for the anaerobic metabolism of skeletal mucsle.

Formation of glucose or glycogen from non-carbohydrate sources is described as gluconeogenesis. The mechanisms involved are essentially the reversal of citric acid cycle and glycolysis. Since some of the reactions are not reversible, those reactions are shortcircuited or substituted by alternate reactions. Any substance which can form any of the derivatives of the citric acid cycle or glycolysis can therefore give rise to glucose or glycogen. Chief among those are the amino acids classified metabolically as glycogenic, lactate and glycerol (from fats). Some of the glycogenic amino acids and the intermediates through which they can form glucose are listed below:

Through Pyruvic acid
Glycine (through serine), alanine
Cysteine, cystine
serine
Methionine

Through Oxaloacetic acid
Aspartic acid

Through Fumarate
Phenylalanine and tyrosine

Through α - ketoglutaric acid
Lysine, Arginine
Ornithine, citrulline
Proline, Hydroxy proline
Glutamic acid
Histidine, Tryptophan

Through Succinyl-CoA
Valine, isoleucine

Methionine

Glucose-alanine cycle

During starvation, the chief amino acid transported from the mucsle to liver is 'alanine'. This is converted to pyruvate (by transmination or deamination) and the pyruvate can be converted to glucose. (For further details, see under the metabolism of alanine in the chapter on protein metabolism).

1. Formation of oxaloacetate: In the reversal of glycolysis, one of the early blocks is in the conversion of pyruvate to phosphoenol pyruvate. Hence this is brought about indirectly by converting pyruvate to oxaloacetate by adding CO_2. This requires an enzyme, pyruvate carboxylase (mitochondrial). In the presence of ATP and biotin it converts pyruvate to oxaloacetate. The details of the reaction have been already considered in the citric acid cycle.

All amino acids which form any of the citric acid intermediates (succinly-CoA, fumarate and α-ketoglutarate) can give rise to oxaloacetate by running through Kreb's cycle. Those which form pyruvate can also form oxaloacetate by the above reaction. Aspartic acid forms oxalocetate directly.

The oxaloacetate formed in the cytosol or the mitochondrion is impermeable to the mitochondrial membrane. Glycolysis and gluconeogenesis occur in the cytosol and citric acid cycle in the mitochondria. Oxaloacetate is an important starting point for either gluconeogenesis or citric acid cycle. The transfer of oxaloacetate across the mitochondrial membrane is brought about by shuttle mechanisms in which citrate, aspartate or malate serve as intermediate substances. The steps are shown in Fig. 14-10. The enzymes required are listed below:

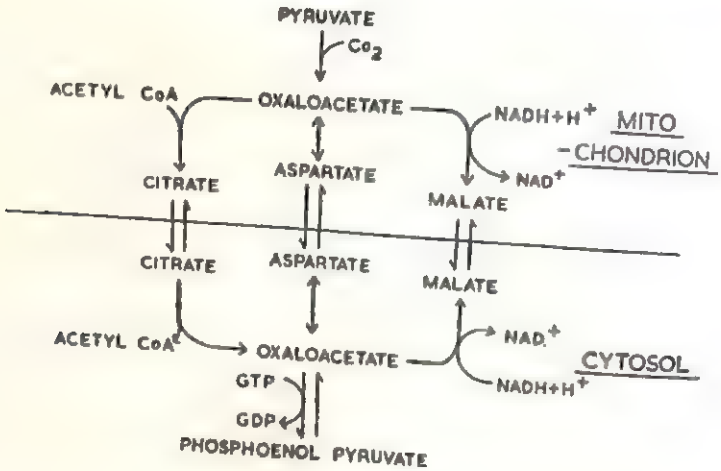
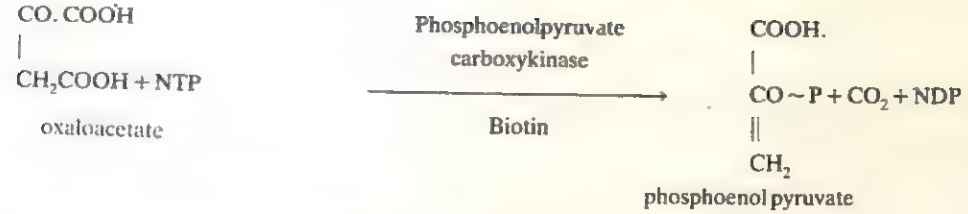


Fig. 14-10.

1. Pyruvate carboxylase
2. Malate dehydrogenase (mitochondrial)
3. Malate dehydrogenase (cytosol)
4. Phosphoenolypyruvate (PEP) carboxykinase
5. Transaminase (mitochondrial)
6. Transaminase (cytoplasmic)
7. Citrate synthetase and
8. Citrate-cleaving enzyme.

2. Formation of phosphoenol pyruvate: The oxaloacetate can now be convert :d to phosphoenol pyruvate by an extramitochondrial enzyme, phosphoenolpyruvate carboxykinase.

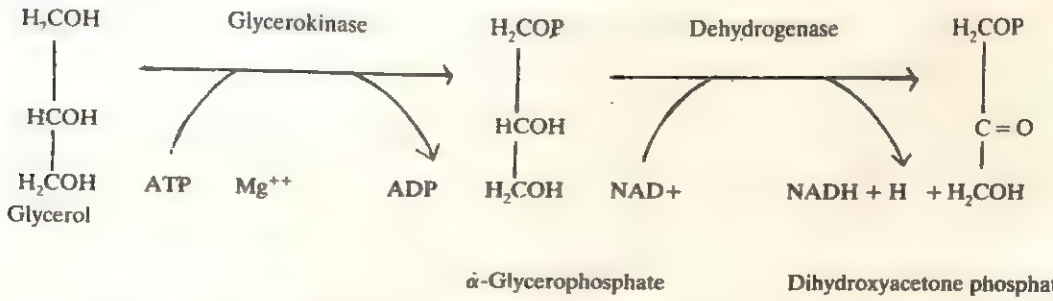


A nucleotide triphosphate (GTP or ITP) serves as phosphate donor. Once phosphoenol pyruvate is formed, all the preceding reactions of glycolysis are reversible upto the formation of fructose-I, 6-Phosphate. This is acted upon by an enzyme fructose-I, 6-diphosphatase which converts it to fructose-6-phosphate. (This enzyme is present in liver, kidney and striated muscle and absent in adipose tissue. Hence gluconeogenesis does not occur in adipose tissue). The fructose-6-phosphate is converted to glucose-6-phosphate by a reversal of the action of phosphohexose-isomerase enzyme. Glucose-6-phosphate can now be converted to glucose-I-phosphate and be synthesized to glycogen by the pathway already considered. Alternately, glucose-6-phosphate may be converted to glucose by the action of glucose-6-phosphatase. This enzyme, as already mentioned, is present in liver and kidney, but absent in muscle. Hence the inability of muscle to add glucose to blood stream directly.

Fructose-I, 6-diphosphatase is an allosteric enzyme. It is strongly inhibited by AMP and stimulated by 3-phosphoglycerate and citrate.

Pyruvate carboxylase activity is promoted by acetyl-CoA which acts as an allosteric modulator. In the catabolic path, the activity of phosphofructokinase is stimulated by AMP and ADP and inhibited by ATP, citrate and $NADH+H^+$. Hexokinase is also inhibited by glucose-6-phosphate and probably also acetyl-CoA and phosphoenolpyruvate. Pyruvate kinase is inhibited by ATP, $NADH+H^+$ and alanine. It is stimulated by fructose-I, 6-diphosphate and glucose-6-phosphate.

Conversion of glycerol to glucose: Glycerol obtained from fats is converted into an active compound, α-glycerophosphate, by the enzyme glycerokinase. The α-glycerophosphate is convertible to dihydroxyacetone phosphate and can thus enter the glycolytic pathway.



Gluconeogenesis is stimulated by adrenal cortical hormones (II-oxycorticoids) with the result there is excessive breakdown of protein and conversion of the carbon skeleton to glucose. The nitrogen part is excreted as urea in the urine. This gives rise to a negative nitrogen balance (more nitrogen excreted in urine than taken in food) and wasting of tissues.

HEXOSE MONO PHOSPHATE (HMP) SHUNT OR DIRECT OXIDATIVE PATHWAY

Pentose Phosphate pathway; Warburg-Dickens pathway

Significance of HMP pathway: This is a pathway which occurs mainly in the liver, adipose tissue, lactating mammary gland, erythrocyte, adrenal cortex and other endocrine glands concerned in the synthesis of steroidal hormones. The oxidation of glucose proceeds here without the intervention of a preliminary glycolysis. The C_1 of glucose is oxidized first whereas in glycolysis it is the C_3 and C_4 which are first oxidized. The coenzyme used in the oxidative reactions of this pathway is $NADP^+$ which is reduced to $NADPH+ H^+$. In glycolysis and citric acid cycle, however, it is $NAD^+ \rightarrow NADH+ H^+$. The reduced $NADPH+ H^+$ is required in the synthesis of fatty acids and cholesterol. Hence the pathway has particular relevance to the sites of these synthesis (1) liver–phospholipids, (2) adipose tissue–fatty acids, (3) endocrine glands–steroid hormones, and (4) lactating mammary gland–milk fat. During the pathway, pentose phosphates are produced. These are necessary for nucleic acid synthesis (ribose and deoxyribose of nucleic acids). A heptose sugar called sedoheptulose is also formed.

The HMP pathway is also called the "SHUNT" pathway, because fructose-6-phosphate can be formed from glucose-6-phosphate by this pathway.

All the enzymes of this pathway are present in the cytoplasm. The pathway does not require the particulate fraction.

The pathway also occurs in the lens and a deficiency of glucose metabolism by this pathway may be associated with some of the cataracts observed (eg:in galactosemia, galactose inhibits glucose-6-phosphate dehydrogenase activity in the lens).

A deficiency of the enzyme glucose-6-phosphate dehydrogenase is noted in the erythrocytes of some persons. They show increased fragility and hypersensitivity to certain drugs like primaquin or ingestion of certain foods like fava beans.

Though the initial reactions are far different from glycolysis, this pathway also is capable of ultimately forming glyceraldehyde-3-phosphate which is metabolized in the usual way to produce energy.

The reactions of the pathway are summarized in Fig. 14-11. The starting point is glucose-6-P formed by hexokinase reaction.

1. Glucose-6-phosphate is dehydrogenated by the action of 'glucose-6-phosphate dehydrogenase'to form 6-phosphogluconolactone. Coenzyme $NADP^+$ is converted to $NADPH+ H^+$. Divalent cations-calcium or magnesium are required as activators.

The enzyme is inhibited by sulphonamides and quinacrine.

2. The lactone is converted to 6-phosphogluconic acid with the addition of a molecule of water by 'gluconolactone hydrolase'.

3. Phosphogluconate dehydrogenase, will now remove two hydrogen atoms from the third carbon to form 3-keto, 6-phosphogluconic acid. One more molecule of $NADP^+$ is converted to $NADPH+ H^+$ in the reaction which also requires calcium, magnesium or manganese ions for activity.

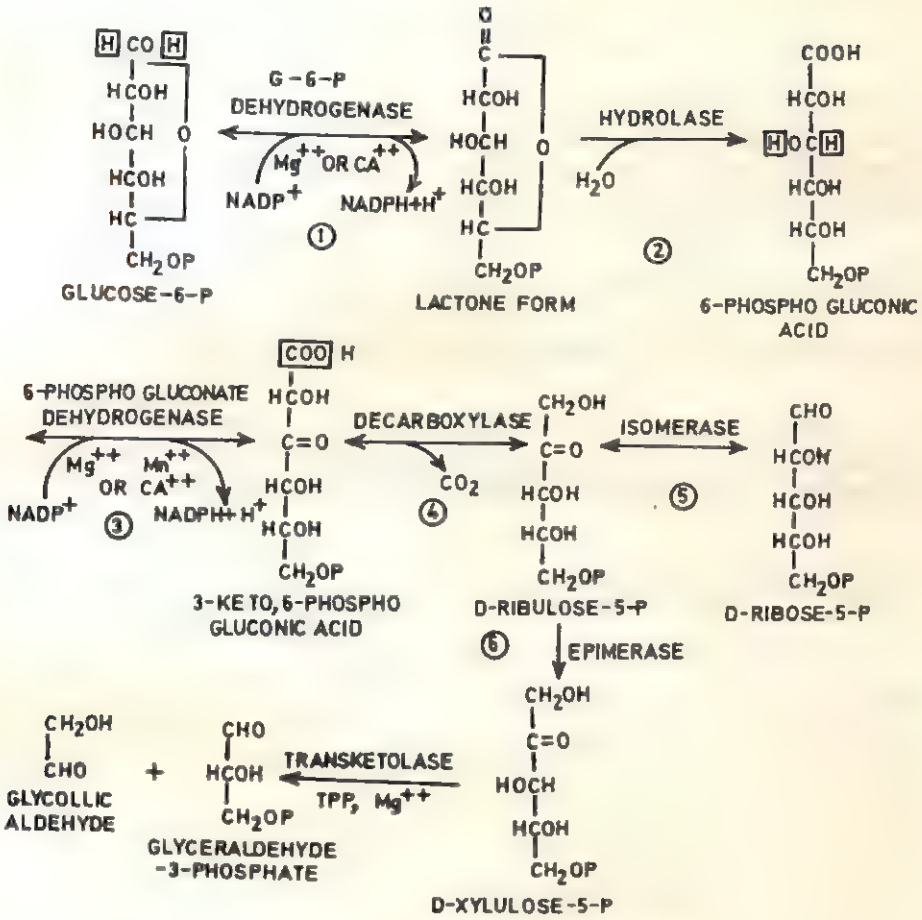


Fig. 14-11. The HMP Pathway

4. The ketogluconic acid now undergoes decarboxylation (of the first carbon) to form D-ribulose-5-phosphate.

5. This can be isomerized to form D-ribose-5-phosphate.

6. It can also be epimerized to form D-xylulose-5-phosphate.

7. D-xylulose-5-phosphate is acted upon by 'transketolase' enzyme which breaks it into a two carbon fragment-glycollic aldehyde, and a three carbon fragment-glyceraldehyde-3-phosphate. The enzyme requires thiamine pyrophosphate (TPP) as coenzyme and magnesium ions as activator.

The two carbon fragment can be added to the pentose to form a heptose - sedo-heptulose-7-phosphate. A 'transaldolase' enzyme will now remove three of the carbons of sedoheptulose in the form of dihydroxyacetone, leaving a tetrose. The dihydroxyacetone can combine with a molecule of glyceraldehyde-3-phosphate to form fructose-6-phosphate.

Uronic acid pathway

This is also a synthetic pathway like the HMP pathway. It provides glucuronic acid and galacturonic acid which are constituents of glycoproteins. Glucuronic acid is also required for detoxication reactions. In the lower animals this pathway also leads to the synthesis of ascorbic acid or vitamin C. The important reactions of the pathway are as shown in Fig. 14-12.

Essential pentosuria is a condition in which there is an inability in the further metabolism of L-xylulose formed from L-gulonic acid. L-Xylulose is hence excreted in the urine. Normally it is reduced to xylitol and through that to D-xylulose which can be utilized.

Certain drugs like barbiturates, chlorobutanol, aninopyrine and antipyrine direct more of glucose into the uronic acid pathway and increase L-ascorbic acid synthesis in experimental animals. In cases of essential pentosuria, they cause increased excretion of L-xylulose.

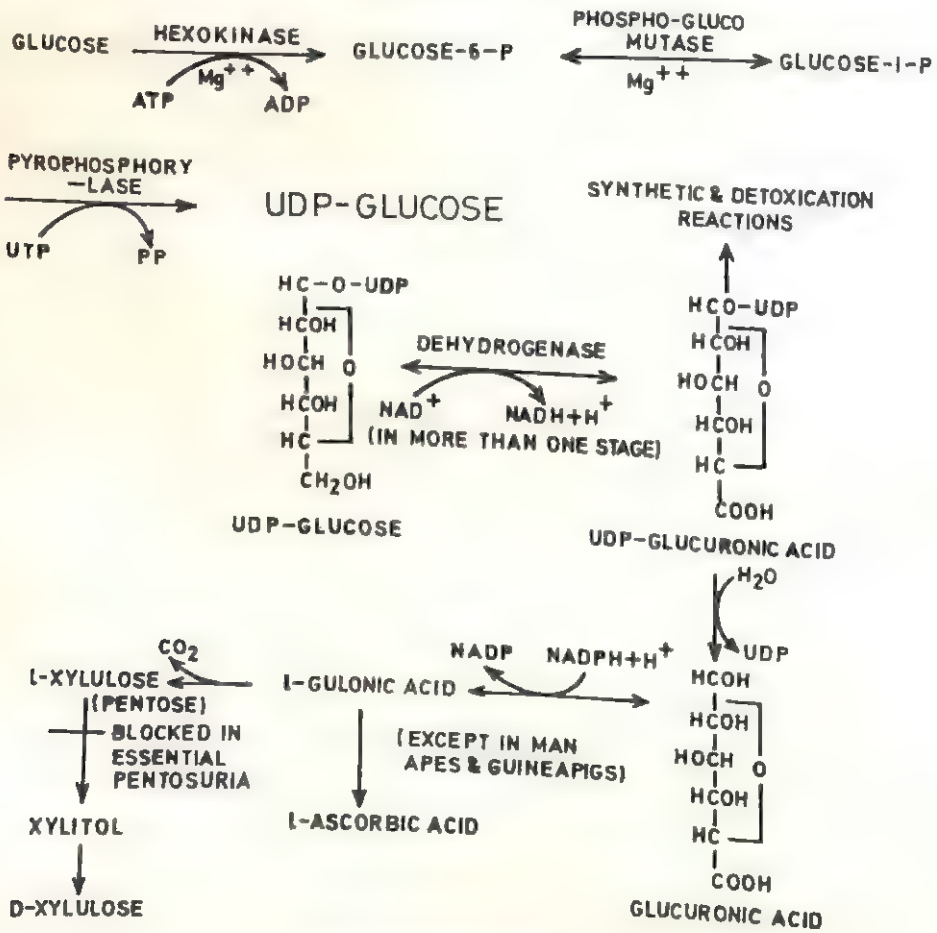


Fig. 14-12. Uronic acid pathway

Metabolism of aminosugars (hexosamines)

N-acetyl glucosamine and glucuronic acid are present in polysaccharide form in the mucopolysaccharides like hyaluronic acid. This is a constituent of vitreous humor, synovial fluid, umbilical cord, skin and bone.

N-acetyl galactosamine and glucuronic acid are present similarly in chondroitin sulfuric acid, a constituent of mucoproteins present in cartilage, blood vessels, tendons and skin.

Glucosamine is formed by a transamidation reaction whereby the C=O of fructose-6-phosphate is converted to CH NH_2. The amido group of glutamine serves as the amino group donor. The glucosamine can undergo epimerization to form galactosamine.

Sialic acids: These are constituents of several glycoproteins. They can be readily synthesized from glucosamine which is acetylated and then epimerized to form n-acetyl mannosamine. This combines with phosphoenolypruvate to form n-acetylneuraminic acid or sialic acid.

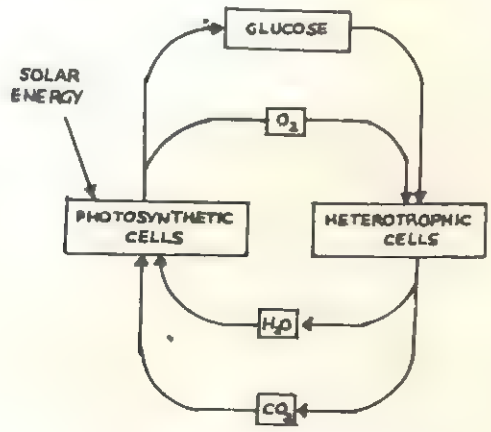
Photosynthesis

Cells can be divided into two classes:

1. Autotrophic (or self-feeding): They can utilize atmospheric CO_2 to synthesize all the organic molecules required by them.

2. Heterotrophic (or feeding on others): They require preformed organic molecules like glucose formed by other cells.

The Carbon-Oxygen Cycle: Living organisms are interdependent. Phostosynthetic cells utilize H_2O and CO_2 and supply glucose and O_2 to the heterotrophic cells. This reciprocal relationship is described as "SYNTROPHY".



CARBON AND OXYGEN CYCLE

The synthesis of carbohydrate from CO_2 and H_2O by the green plant is called photosynthesis. The energy for the synthetic reaction is derived from the radiant energy of sunlight, which is captured and converted to chemical energy as a first step (light reaction). The chemical energy is then utilized for the synthesis of carbohydrate and other substances (dark reaction).

The chlorophyll of plants is the main architect of the process. It is present in particles known as 'grana' which are contained in disc shaped structures called 'chloroplasts' Chlorophyll has a heme like structure; at the centre of the tetrapyrrole structure, chlorophyll has magnesium instead of iron. There are also differences in the side chains. A long chain alcohol called 'phytol' is attached by ester linkage to the propionic acid of ring IV. 'Chlorophyll-A' which is the major component has a methyl group in ring II in position 3, whereas 'chlorophyll-B', a minor component, has an aldehyde group in that position, (See Fig. 14-13.). Other plant pigments like beta carotene and phycobilins of the algae which have an open tetrapyrrole structure as in bile pigments may also play a role in radiant energy fixing reactions.

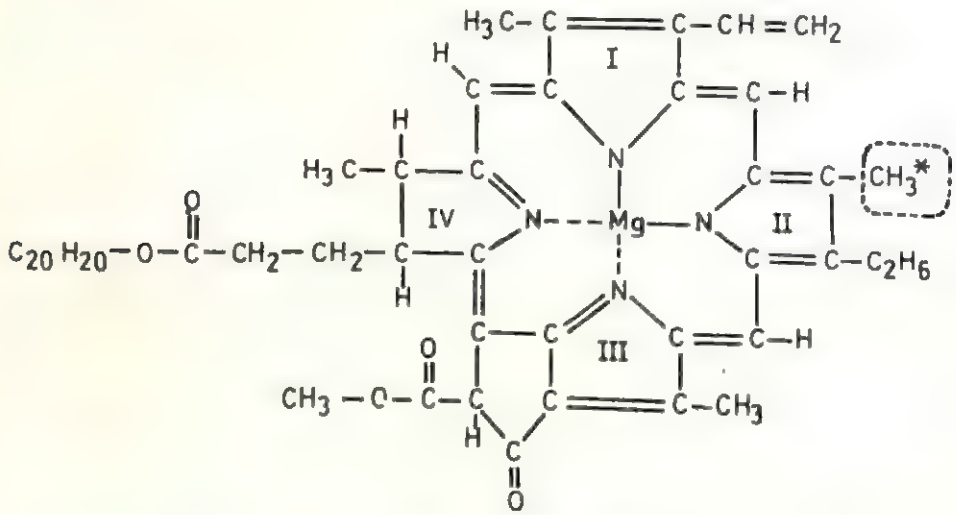


Fig. 14-13. Chlorophyll-A

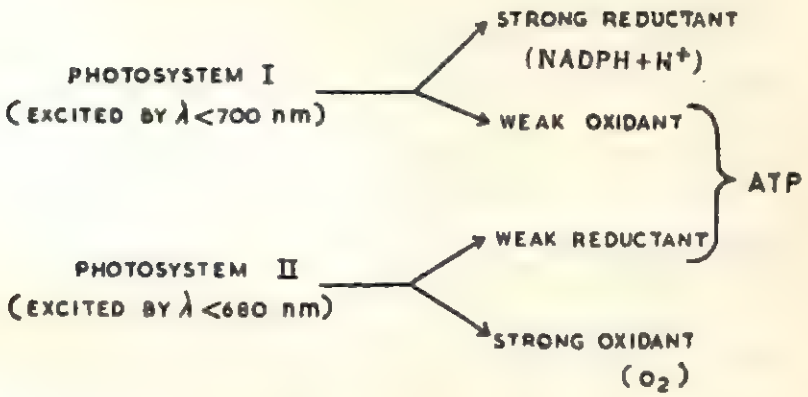
The Hill reaction: When chlorophyll molecule is subjected to the incident light radiations, some of the electrons in its molecule get excited and loosened from the rest of the molecule, thus raising the energy level of the molecule. Photosynthesis requires two kinds of photosystems.

Photosystem I: This is excited by wavelengths shorter than 700 nm. It is primarily concerned in the production of a "reductant" which leads to the formation of $NADPH+H^+$

Photosystem II: This is excited by wavelengths shorter than 680 nm and produces the oxidant leading to the formation of O_2.

In addition, photosystem I also produces a weak oxidant and photosystem II a weak reductant. The interaction between the weak reductant and the weak oxidant will result in a flow of electrons from photosystem II to photosystem I and the electron flow leads to the generation of ATP. This is called *"photophosphorylation"*.

The photosystem I acts through an intermediate substance called *"ferredoxin"* a non-heme iron protein of low molecular weight. The iron acts as an electorn carrier between the chlorophyll and $NADP^+$.



The overall reactions is:

$$2\,NADP^+ + 2H_2O \longrightarrow 2NADPH + 2H^+ + O_2$$

The Dark Reaction: CO_2 fixation: It is so called because the reaction can take place in the absence of light. Calvin and associates investigated the reaction in detail. ^{14}C labelled carbondioxide is rapidly incorporated into ribulose-I, 5-diphosphate to form two molecules of 3-phosphoglyceraldehyde, which can, by a reversal of glycolysis form a glucose molecule. A continuous supply of ribulose-I, 5-diphosphate is maintianed by a simultaneous hexosemonophosphate shunt pathway. To fix up six molecules of CO_2 (an equivalent of one glucose molecule) it requires 6 moelcules of the pentosediphosphate to run through the above cycle. More than half of all photosynthesis on this planet is carried out by the microscopic algae, diatoms and other inhabitants of the oceans which are all classed as 'PHYTOPLANKTON'. Some of the photosynthetic bacteria are strict anerobes and use only H_2S as hydrogen donor instead of H_2O.

$$2\,H_2S + CO_2 \xrightarrow{\text{light}} CH_2O + H_2O + 2\,S$$

Oxygen is poisonous to such bacteria. Substances like isopropanol and ethanol can also serve as hydrogen donors in photosynthesis by certain organisms.

Fermentation by Yeast

The enzymes of yeast and a few other microorganisms bring about the same glycolytic reactions as in mammalian tissues to form pyruvate. Some of the pyruvate formed is converted to ethyl alcohol as follows–

$$CH_3CO\,COOH \xrightarrow[\text{pyruvic decarboxylase}]{TPP,\ Mg^{++}} (CH_3CHO.TPP)$$

active acetaldehyde

$$\longrightarrow CH_3CHO + TPP$$

$$CH_3CHO \xrightarrow[\text{alcohol dehydrogenase}]{NADH + H^+\ NAD^+} CH_3CH_2OH$$

The reaction $NADH + H^+ \longrightarrow NAD^+$ is linked to the phosphoglyceraldehyde dehydrogenase reaction in glycolysis (compare with the lactic dehydrogenase function).

Metabolism of ethyl alcohol: Alcohol metabolism takes place almost exclusively in the liver and yields as much as 7 calories per gram of alcohol.

$$CH_3CH_2OH \xrightarrow[\text{alcohol dehydrogenase}]{NAD^+\quad NADH+H^+} CH_3CHO$$

$$CH_3CHO + HS\,CoA \xrightarrow[\text{aldehyde dehydrogenase}]{NAD^+\quad NADH+H^+} CH_3COS\,CoA$$

The acetyl-CoA is oxidized in the citric acid cycle.

Pasteur effect: During yeast fermentation, Pasteur observed that the rate of fermentation varied inversely as the availability of oxygen. Under anaerobic conditions more of alcohol is produced from pyruvate, whereas under aerobic conditions, the pyruvate is directly converted to acetate and oxidized in the citric acid cycle. This is described as the 'Pasteur effect'.

In mammalian tissues also, availability of plenty of oxygen enhances the reactions of citric acid cycle and leads to a retardation of the glycolytic reactions. There are only limited quantities of ADP and inorganic phosphate in the tisues. These are converted to ATP in the aerobic process and there is a deficiency of these substrates to feed glycolytic reactions. Reduced ADP and AMP levels in the tissues also inhibit phosphofructokinase, the rate-limiting enzyme in glycolysis.

Crabtree Effect: This can be considered to be the reverse of the Pasteur effect. High concentrations of glucose inhibit citric acid cycle reactions and enhance gycolysis. In this case, the limited amounts of inorganic phosphate and NAD^+ are used up for glycolysis. Citric acid cycle and oxygen consumption are decreased.

Carbohydrate metabolism in the erythrocyte: The stroma consists of about 50% protein and 10% lipid, mainly phospholipid. There are no nuclei and no mitochondria. There is also no glycogen. But the erythrocytes have a fairly high rate of metabolism and utilize 1.5 to 2.2 m. moles of glucose per hour per litre of erythrocytes which is equal to abour 25 calories of energy. This much energy is required for pumping out sodium and pumping in potassium, and for keeping the hemoglobin in the reduced state.

Glycolysis accounts for about 80% of glucose metabolism in the erythrocyte. The remaining 20% of glucose is oxidized by the H.M.P. pathway. There is no citric acid cycle (since there are no mitochondria). The reduced NADPH+ H^+ formed is utilized for keeping the erythrocyte glutathione in the reduced state.

$$NADPH + H^+ + GS\text{-}SG \xrightarrow{\text{GSH reductase}} 2GSH + NADP^+$$

The GSH may play a role in keeping hemoglobin in the reduced state.

A defici. y of the enzyme glucose-6-phosphate dehydrogenase occurs as an inborn error of metabolism in some individuals. They are much prone to the action of drugs like primaquin (antimalarial) and toxins present in fava beans which produce hemolysis in such subjects. The hemolysis may be a result of production of H_2O_2 which cannot be promptly removed due to the deficiency of glucose-6-phosphate dehydrogenase and the resultant lack of NADPH+ H^+

The erythrocyte has about one hundred enzyme systems, all of which including the cell proteins themselves are formed during the preerythrocyte (nucleated) stages. The mature erythrocyte can no longer synthesize any enzyme or protein since it has no nucleus.

The Rapoport-Luebering Cycle:

The erythrocyte metabolizes excessive amounts of glucose in the glycolytic pathway. This will generate much of ATP which is not required and cannot be used by the erythrocytes. ATP production by substrate phosphorylation from 1,3-diphosphoglycerate is prevented in the erythrocyte by taking a diversion pathway shown in fig. 14-14. This pathway is known as Rapoport-Luebering Cycle. It not only prevents accumulation of ATP,but also supplies DPG which is required in large amounts for the hemoglobin function.

DPG, like CO_2, exerts profund effects on the carriage of O_2 by red cell hemoglobin. In the deoxygenated form, the four chains of hemoglobin are relatively tightly bound by several non-covalent bonds. The last but one amino acid in all the four chains is tyrosine. This portion of the chains forms the hydrophobic pocket. A molecule of 2, 3-DPG combines by salt bridges with 4 cationic groups in the beta chains. further adding to the tight binding. In the deoxygenated hemoglobin, 2, 3-DPG is present in the same molar concentration as hemoglobin itself. During oxygenation, the 2, 3-DPG is released from hemoglobin, the salt bridges between the four peptide chains are broken and the four tyrosine residues get released from their hydrophobic pockets. These changes occur gradually and thus contribute to the sigmoid shape of the oxygenation curve.

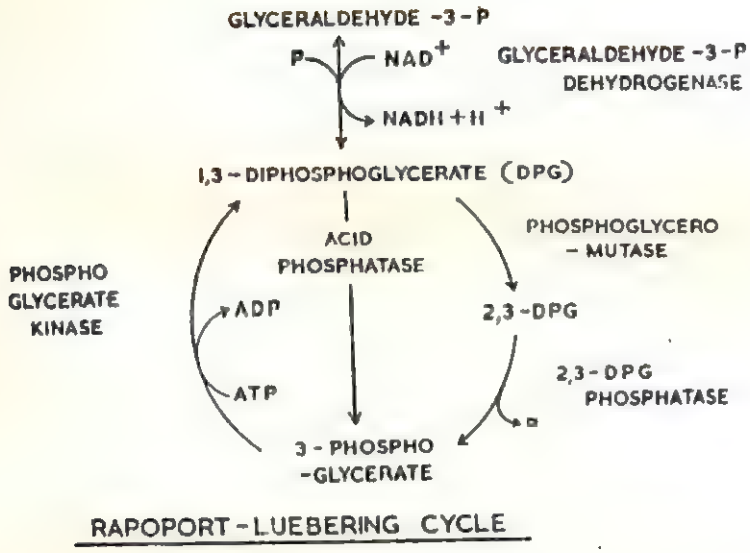


Fig. 14-14

Metabolism in Cancer:

Cancer cells show nearly same oxygen consumption as normal cells, but 5 to 10 times as much glucose consumption. There appears to be some difficulty in integrating glycolysis followed by citric acid cycle. Even in the presence of enough oxygen glucose metabolism is mainly by glycolysis (hence called 'aerobic glycolysis') and produces large amounts of lactic acid. The lactate enters the systemic blood and is reconverted to glucose in the liver. The conversion of glucose to lactate in glycolysis produces only 2 ATP whereas the conversion of lactate to glucose in the liver requires 6 ATP. Hence the cancer cells live a parasitic life and drain off much energy from the host organism.

References:

Dickens, F., Randle, P.J., Whelan, W.J.(eds):
Carbohydrate Metabolism and its Disorders. Acad. Pr., N.Y., 1968
Gergely, J. (ed): Biochemistry of Muscle Contraction., Little Brown., Boston., 1964.
Goodwin, T.W.(ed.): Biochemistry of Chloroplasts., Acad. Pr., N.Y., 1966.
Goodwin,T.W. (ed.): Metabolic Role of Citrate, Acad. Pr., N.Y. 1968.
Eisensteine, A.B.(ed.): Biochemical Aspects of Hormone Action,Little Brown, Boston 1964.
Hoffman, W.S.:
The Biochemistry of Clinical Medicine 4th ed., Yearbook Med. Pub., Chicago., 1970
Hillman, S. and Touster, C.: Non-glycolytic Pathways of Metabolism of Glucose., Acad. Pr., N.Y., 1964.
Hsia. D.Y.: Inborn Errors of Metabolism.,2nd. ed., Yearbook Med., Pub., Chicago., 1966.
Kamen, M.D.: Primary Process in Photosynthesis Acad. Pr. N.Y., 1963.
Rodahl, K. and Horvath. S.M. (eds):Muscle as a Tissue., McGraw-Hill., N.Y., 1962.
Soskin, SBand Levin, R.: Carbohydrate Metabolism, 2nd ed., Univ. Chicago Pr., 1952.
Stanbury, J.B., Wyngaarden, J.B. and Fredrickson, D.S.:
The Metabolic Basis of Inherited Disease,2nd. ed., McGraw-Hill.,N.Y., 1966.
Szent-Gyorgi, A.: Chemistry of Muscular Contraction., Acad. Pr., N.Y., 1961.
Vernon, L.P. and Seely, G.R.(eds.): The Chlorophylls, Acad. Pr., N.Y., 1966.
Whelan, W.J. (ed.): Control of Glycogen Metabolism. Acad. Pr., N.Y., 1968.

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LIPID METABOLISM

THE metabolism of lipids comprises the metabolism of neutral fats or triglycerides, phospholipids, sterols and others. Weight per weight, the triglyceride supplies twice the amount of energy compared to carbohydrates and proteins. Further it can be consumed in diet in an almost pure form and can be stored in the body as almost 90% pure fat. Hence it is the best form of obtaining and storing energy. It is stored in the body in certain sites known as fat depots. The subcutaneous tissues, intermuscular, perinephric, omental and mesenteric tissues store neutral fat in their adipose tissues and may contain about 6,000 grams fat in a 70 kg. man. The lipid of these tissues is mainly neutral fat. Brain and nervous system in large amounts and all other tissues in smaller amounts contain phospholipids, glycolipids, cholesterol and others — the total reaching about 3,000 grams. The liver contains about 20 grams neutral fat, 50 grams phospholipid and 5 grams cholesterol. The blood and body fluids contain a total of 75 grams of the different lipids. Thus there is nearly 10 kg. of lipid in a 70 kg. man. The lipid in the adipose tissue is dependent on the bulk of this tissue and may reach levels much higher than 6,000 grams in the obese individual or may remain quite low in a thin individual.

Apart from its function of storage of energy the subcutaneous adipose tissue also helps in giving the smooth rounded contours to the body and also insulates against changes in the environmental temperature and prevents heat loss.

Fat in the diet is essential to supply more calories without increasing the bulk of the food. It is also necessary to supply the fat-soluble vitamins and certain polyunsaturated fatty acids (essential fatty acids) which cannot be synthesized in the body.

Dietary fat, after digestion, is absorbed mainly by the lymphatics in the form of chylomicrons which are minute droplets of fat (0.5μ diameter) enveloped by a thin layer of lipoprotein. Cholesterol and long chain free fatty acids are also present. Short chain fatty acids (C-12 and below) and phospholipids are absorbed through the portal blood stream and reach the liver.

Plasma lipids: In the postabsorptive state, the blood plasma contains about 550 mg. of lipids which are distributed as follows:-

Lipids of the Blood Plasma in Man

Lipid	mg/100 ml	
	Mean	Range
Total lipids ..	570	360–820
Triacylglycerol ..	142	80–180*
Total phospholipid† ..	215	123–390*
Lecithin ..		50–200
Cephalin ..		50–130
Sphingomyelins ..		15–35
Total cholesterol ..	200	107–320
Free cholesterol (nonesterified) ..	55	26–106
Free fatty acids (nonesterified) ..	12	6–16*

Total fatty acids (as stearic) range from 200-800 mg/100 ml; 45% are triacylglycerols, 35% phospholipids, 15% cholesteryl easter, and less than 5% free fatty acids.

* Varies with nutritional state.

† Analyzed as lipid phosphorous; mean lipid phosphorous — 9.2 mg/100 ml (range 6.1-14.5). Lipid phosphorous X 25 = phospholipids as lecithin (4% phosphorous).

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Of the phospholipids, lecithins and cephalins predominate and only small amounts of sphingomyelins are present. The cholesterol exists in two forms — the ester form which averages about 145 mg/100 ml and the free form which forms about 55 mg/100 ml.

Transport of lipids in plasma: Lipids being water-insoluble, special mechanisms are required for their transport in plasma. Chylomicrons are an important mode of transport for plasma lipids. The phospholipid-protein covering around the fat droplet helps in preventing the drops from coalescing and keeps them in suspension. Lipoproteins with varying lipid-protein concentration also occur in plasma independent of the chylomicrons. They are mostly derived from liver. Free fatty acids (FFA) or nonesterified fatty acids (NEFA) are derived by intestinal absorption or by release from adipose tissue. They form a loose complex with plasma albumin and are transported along with that protein.

Two different methods of analysis are employed in the study of plasma lipids.

1. Electrophoresis: The procedure is similar to electrophoresis of plasma proteins and uses different supporting media like agar, cellulose acetate or filter paper. The lipid pattern may be developed by using suitable stains like Congo Red, Sudan Black or oil Red-O, and the bands so obtained are called the *a*, *β* etc., lipoproteins. The fractions may be eluted with suitable solvents and their chemical composition determined.

2. Ultracentrifugation: Ordinary laboratory centrifuge produces centrifugal forces of 500 to 4,000 times gravitational force (x g). The centrifugal force depends on the radius of rotation and the speed of rotation. The radius remains the same for any given instrument, but the speed can be altered.

Using sufficiently high speeds (30,000 to 100,000 R.P.M.) even colloidal particles like the protein molecules can be separated out according to their molecular weights. The solution of a mixture of proteins in an appropriate buffer is subjected to ultracentrifugation. The proteins are forced towards the periphery (*i.e.* bottom; of the centrifuge tube and areas within the column of liquid get packed with the protein molecules, the heavier ones nearer the bottom and the lighter ones farther from the bottom. When a state of equilibrium is attained, the pattern of this packing can be studied by an optical system — the Schlieren lens system. The areas packed with protein molecules will show a change in the refractive index at that area in the column and this will be recorded on a photographic plate as a peak. The area of the peak will be proportional to the concentration of protein at that point. The sedimentation rate of each protein is a characteristic of its own and is expressed as Svedberg units. A Svedberg unit indicates sedimentation at the rate of $1x10^{-10}$ centimeter per dyne per second per gram of the material.

The technique of ultracentrifugation can also be used for substances which float *i.e.*, substances which are lighter than the suspension medium, e.g. lipoproteins. The plasma is suspended in a saline solution of high specific gravity (1.063) and subjected to centrifugation in an ultracentrifuge. At this specific gravity, the lipoproteins and lipids tend to float to the surface while the protein tends to sink. The degree of flotation of each component is expressed in terms of S*f* units (Svedberg flotation units). The fractions of different S*f* values can be collected and analyzed. The composition of the lipid fractions by the two methods is given in table 15-1. It may be seen that, in general, the total lipid, triglyceride and cholesterol content decrease while the protein content and to some extent also the phospholipid content increase as the density of the fraction increases or the S*f* value decreases.

Plasma Lipoproteins

The protein component: The protein components of plasma lipoproteins are called the 'apoproteins' and are classified into three major groups — A, B and C.

The A group are present mainly in HDL (or the alfa lipoprotein). The B group are present in LDL (beta lipoprotein) mainly and also in VLDL (prebeta lipoprotein) and the chylomicrons. Apoprotein C are small polypeptides found in HDL, VLDL and

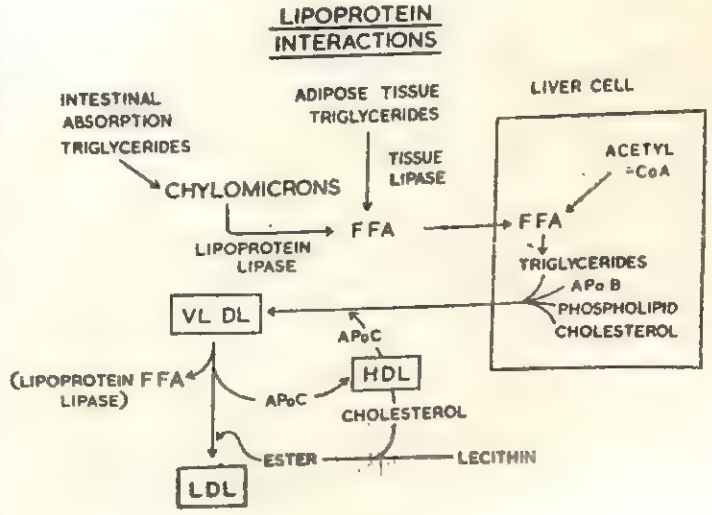


TABLE 15-1

Plasma Lipoproteins

Name of fraction (ultracentri-fugation)	Turnover time	Electrophoretic fraction	Density	Sf value	Diameter of molecules nm	mg/100 ml plasma	PERCENTAGE COMPOSITION					
							protein	phospho-lipid	cholesterol free	cholesterol ester	T.G.	F.A.
Chylomicrons	5 mins.	origin	< 0.95	> 400	300 - 5000	100 - 250	2	7	2	6	83	—
VLDL	2 hrs.	prebeta	0.95 - 1.006	12 - 400	300 - 750	130 - 200	9	18	7	15	50	1
LDL	4 days	beta	1.006 - 1.063	0 - 12	200 - 250	210 - 400	21	22	8	38	10	1
HDL	10 hrs.	alfa	1.063 - 1.210	—	100 - 150	50 - 130	33	29	7	23	8	—
VHDL	10 hrs.	alfa	> 1.21	—	100	290 - 400	57	21	3	14	5	—

VLDL: very low density lipoprotein
LDL: low density lipoprotein
HDL: high density lipoprotein
VHDL: very high density lipoprotein
T.G.: triglycerides
F.A.: free fatty acids

chylomicrons. The apolipoproteins — apoproteins — are usually referred to in an abbreviated form as Apo-A, Apo-B and Apo-C.

Chylomicrons: These are mainly of intestinal origin and their quantity is increased in plasma following a fatty meal. The plasma becomes opalescent. The protein component is mainly Apo-B. They are rapidly taken up from blood by the adipose tissue, heart and skeletal muscle. Lipoprotein lipase or 'clearing factor', an enzyme located in the capillary walls of these tissues, hydrolyzes the lipids of the chylomicrons and helps their passage into the tissues. The enzyme is activated by heparin and a component of Apo-C called Apo-CII. Glucose and insulin enhance its activity. Epinephrine, ACTH, growth hormone and corticosteroids inhibit. The effect of these hormones is mediated through cyclic AMP. 90% of the chylomicron lipid is removed this way. The chylomicrons still contain cholesterol and cholesterol esters and are now taken up by the liver.

VLDL (Very Low Density Lipoproteins): The bulk of VLDL is secreted by the liver in a manner similar to chylomicrons by the intestines. The protein component in them is also mostly Apo-B. The fatty acids are derived from hepatic synthesis from acetyl-CoA (derived from carbohydrate) in a well fed individual and from the fatty acids coming from peripheral tissues through plasma (plasma FFA) in an ill-fed individual. The fatty acids are incorporated into triglycerides and then into VLDL. The clearing factor acts on VLDL in a manner similar to its action on chylomicrons and helps in removing the fatty acids from them.

LDL (Low Density Lipoproteins): They seem to be a product of degradation of VLDL and chylomicrons. This fraction is rich in cholesterol and is taken up by all cells including liver cells and metabolized. The LDL are taken up in toto at specific binding sites on the cell surfaces.

HDL (High Density Lipoproteins): These are synthesized and secreted by both the liver and the intestinal mucosa. The HDL secreted by intestines contain only Apo-A, whereas those from Liver contain Apo-C also. The lipids are mainly cholesterol and phospholipids. An enzyme, 'lecithin: cholesterol acyltransferase' (LCAT), transfers a fatty acid from lecithin to cholesterol and forms cholesterol ester and lysolecithin. The cholesterol ester then gets transferred from HDL to VLDL and LDL.

ROLE OF THE LIVER IN LIPID METABOLISM

The lipid content of liver is about 5%. The phospholipids and short chain fatty acids absorbed via the portal blood are brought to the liver. It also takes up the free fatty acids from systemic blood, and the triglycerides from the chylomicrons. The latter are hydrolyzed to fatty acid and glycerol. Liver is the main source for endogenous plasma lipoproteins. Fatty acids taken up from circulation and fatty acids synthesized in the liver are incorporated into VLDL and secreted back into circulation. High carbohydrate diets (particularly diets rich in sucrose and fructose), high FFA levels in plasma, ethanol and insulin favour enhanced synthesis of triglycerides and VLDL by the liver.

It also synthesizes cholesterol and supplies to the blood. The quantity of cholesterol synthesized is regulated to keep the total amount available from food and by hepatic synthesis constant. Liver is also concerned in the conversion of cholesterol to bile acids and its elimination in bile salts. Thus it plays an important role in homeostasis of blood cholesterol.

Fatty livers and lipotropic substances: A rise in the lipid content of liver much beyond the usual 5% is an abnormal condition and is described as fatty liver. The causes for fatty liver can be discussed mainly in four groups — (1) Increased mobilization of fat from depots to liver. (2) Impaired utilization of fat in the liver and impaired transport from liver. (3) Hormonal causes. (4) Toxic causes.

1. Increased mobilization of fat from depots to liver: This occurs in conditions of carbohydrate deprivation when fat becomes the principal source of calories. The fatty livers of diabetes mellitus and starvation are of this type. Here the liver is only serving a physiological function to an excess and is otherwise normal. Hence they are also called 'physiological fatty livers.'

2. Impaired utilization of fat in the liver and impaired transport of fat from liver: In this type of fatty liver, there is a metabolic block in the production of plasma lipoproteins. In choline deficient animals, plasma VLDL are virtually absent. Choline is taken in diet as such or can be formed in the body if certain other substances are available. These include protein rich diets to supply the amino acids serine and methionine; threonine as an essential constituent of hepatic enzymes, folic acid and B_{12} to supply methyl groups for choline synthesis.

Essential fatty acids are required for the synthesis of phospholipids. Deficiency of pyridoxine and inositol, vitamin E and a selenium containing factor called 'Factor 3' also induce fatty livers.

Substances like choline, methionine and others which prevent fatty livers are called 'lipotropic substances.'

Fatty livers can be induced in experimental animals by feeding them large amounts of cholesterol. It competes with the available supply of essential fatty acids and diverts them for its own esterification, thereby causing a deficiency of essential fatty acids.

Ethionine, the ethyl analogue of methionine, induces fatty liver by competing with ATP for the formation of S-adenosylethionine instead of S-adenosylmethionine (active methionine). Transmethylation reactions are hence interrupted.

3. Hormonal causes: Diabetes mellitus as a cause for fatty liver has been already mentioned. Injections of anterior pituitary hormone, due to its 'ketogenic' factor, will cause mobilization of large amounts of lipid from tissues to liver and cause a fatty liver. The pancreas is said to contain a hormone 'lipocaic' which is a lipotropic substance. But this view is no longer valid. The lipotropic effects of raw pancreatic juice may be on account of the digestive enzymes which promote protein digestion and make methionine available or due to the choline and inositol contained in the pancreatic extracts.

4. Toxic factors: Carbon tetrachloride impairs protein synthesis and also the conjugation of protein with lipid. The secretion of lipoprotein by the liver cell is thus impaired.

Alcoholism is an important cause of fatty livers. Alcohol dehydrogenase requires NAD^+. Diversion of NAD^+ for this purpose causes slowing down of beta oxidation which also requires the coenzyme. Citric acid cycle also is slowed down. Plasma free fatty acids are increased. The increased levels of $NADH + H^+$ cause conversion of more of pyruvate to lactate. The excretion of larger amounts of lactate in urine results in a competitive decrease in uric acid excretion and symptoms may be aggravated in gouty subjects.

ROLE OF ADIPOSE TISSUE IN LIPID METABOLISM

Adipose tissue has a function in storage of lipid and its supply to tissues as needed in a manner very similar to the storage of glycogen in liver in carbohydrate metabolism. The free fatty acids absorbed from gastrointestinal tract and the fatty acids derived from triglycerides of chylomicrons (intestinal absorption) and lipoproteins (from liver and intestines) after their release by hydrolysis by lipoprotein lipase — are all taken up by the adipose tissue and resynthesized into triglyceride and stored. The carbohydrate (glucose) also is converted by adipose tissue into fatty acids and stored as triglyceride. These two processes — uptake of fatty acids and glucose from blood and their synthesis into triglyceride — are enhanced by the action of the hormone insulin.

The depot fat of each species is fairly constant in composition and has a melting point close to the body temperature of that species. The lipid in the depots is hence in a liquid state. The composition can however be altered by alterations in the dietary lipids for long periods. Feeding animals (pigs for example) with diets rich in vegetable fats (low melting point) such as groundnut oil results in deposition of a fat with melting point lower than normal. Feeding carbohydrate-rich diets like cereals will lead to deposition of solid fat with high melting point, since the fatty acids synthesized from carbohydrates are of the saturated long chain type. This is used in the pork industry.

The triglycerides are constantly broken down into fatty acids and liberated into the plasma to meet the energy requirements of the several tissues. They are transported in the plasma by forming a loose complex with plasma albumin. Several hormones — epinephrine, norepinephrine, glucagon, ACTH, growth hormone and thyrotropic hormone — stimulate the release of fatty acids from adipose tissue.

Triglyceride synthesis and lipolysis do not follow the same pathway in the adipose tissue. The synthesis is intimately linked with glucose metabolism. The alfa-glycerophosphate which has to be esterified with fatty acids to form triglyceride can be formed by one of two pathways — (1) activation of glycerol which has been liberated by action of lipoprotein lipase or other lipases by the enzyme 'glycerokinase' or (2) reduction of dihydroxyacetone phosphate by the enzyme 'glycerophosphate dehydrogenase'. Adipose tissue does not possess glycerokinase enzyme. Hence it has to depend on the production of dihydroxyacetone phosphate by glycolysis.

Insulin and glucose stimulate triglyceride synthesis by supplying adequate amounts of alfa-glycerophosphate for re-esterifaction of the fatty acids taken up from blood. They also stimulate lipogenesis from glucose via acetate and further provide reduced NADPH + H^+ for the fatty acid synthesis by stimulating oxidation of glucose by the HMP-pathway.

Lipolysis in adipose tissue is brought about by a 'hormone-sensitive lipase' distinct from lipoprotein lipase. As in glycogenolysis, the hormonal influence on lipolysis seems to be mediated by cyclic AMP levels in that tissue. The epinephrine group of hormones, by stimulating adenyl cyclase to produce more of cyclic AMP from ATP, favour the conversion of inactive form of the hormone-sensitive lipase to an active form. Caffeine accentuates the action of these hormones by inhibiting the breakdown of the cyclic AMP by '3,5-nucleotide phosphodiesterase'. The result is increased lipolysis in adipose tissue and increase of plasma FFA (free fatty acids).

Insulin tends to lower cyclic AMP levels by inhibiting adenyl cyclase and stimulating the phosphodiesterase. Nicotinic acid and prostaglandin E_1 accentuate the insulin effect. The FFA level of plasma is lowered as a result of decreased lipolysis in the adipose tissue due to inhibition of the hormone-sensitive lipase.

Brown Adipose Tissue: While the adipose tissue in adult mammal including human is of the type described above (which may be called 'white adipose tissue'), a second type of adipose tissue with an abundant blood supply and nerve supply is seen in the new-born animals (including human), and in the hibernating animals. The tissue occurs in masses around the neck and in the inter-scapular region in the back, below the subcutaneous white fat. The cells have granular cytoplasm with numerous fat droplets and round nuclei. The granularity of the cytoplasm is on account of the high concentration of cytochromes. The brown adipose tissue has a higher metabolic activity and is capable of producing larger amount of heat. The oxidation of fatty acids is somehow not coupled with phosphorylation. The energy produced is converted to heat energy and raises the body temperature. On exposure of the animal to cold, noradrenaline is released in large quantities. This activates the adipose tissue lipase to release free fatty acids for oxidation and heat production. Brown adipose tissue disappears during the growth of human child and is altogether absent in the adult man.

Plasma free fatty acids (FFA) or non-esterified fatty acids (NEFA): In the post-absorptive state, plasma contains 6-16 mg. of FFA per 100 ml. Most of these are transported in the plasma by forming a loose complex with plasma albumin. A small amount is also associated with high density lipoprotein.

The plasma FFA, mainly derived from adipose tissue in the postabsorptive state, are transported to the liver and other tissues for their utilization. Though apparently small in quantity compared to other lipid fractions in the plasma, they exhibit a very rapid turnover rate with a half-life of only 1 to 3 minutes, showing that they are rapidly taken up by the tissues and metabolized. Administration of glucose or insulin decreases plasma FFA levels while the administration of epinephrine, norepinephrine or growth hormone increases them. Adrenal cortical hormones and thyroid hormones enhance the latter action. They are also increased in diabetes mellitus and starvation.

Adipokinin, secreted by the anterior pituitary, also enhances the plasma FFA levels by increasing lipolysis of depot fats.

The FFA are utilized in the liver mainly for their incorporation into very low density lipoproteins (VLDL) which are returned to the plasma. Some are oxidized to acetate by beta-oxidation. The acetate may be further oxidized in the citric acid cycle or utilized for synthetic reactions, mainly cholesterol synthesis. Some amount of acetate is also converted to acetoacetate and returned to plasma.

Other tissues including skeletal and cardiac muscle can take up FFA from circulation and utilize them for energy production. In fact, when FFA and acetoacetate are available in sufficient quantities, they prevent the entry of glucose into skeletal muscle and are utilized by that tissue in preference to glucose. The accumulation of glucose in blood due to diminished uptake by skeletal muscle will, in turn, suppress lipolysis in the adipose tissue and decrease the FFA release. This may be a direct effect by making available alfa-glycerophosphate for re-esterification of the fatty acids or indirect effect through the increased blood glucose levels stimulating insulin production. This inter-relationship between FFA and glucose levels in plasma and their uptake and utilization by adipose tissue and other tissues is referred to as 'glucose-fatty acid cycle' (Randle *et al;* 1963).

Brain is a notable exception in that it can utilize only glucose for energy requirements under all conditions.

An overview of lipid metabolism in different tissues is presented schematically in fig. 15-1.

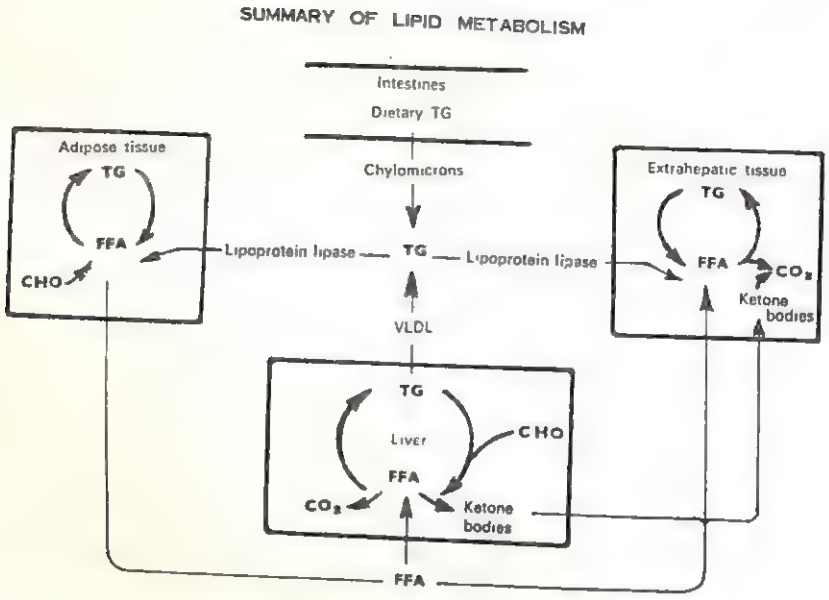


Fig. 15.1 An over-view of lipid metabolism

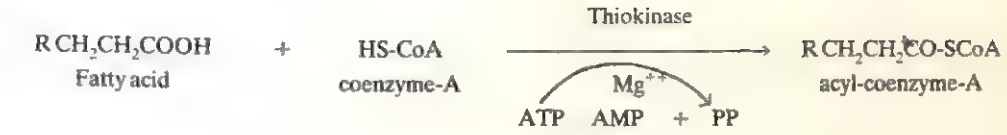
TG = triglycerides
CHO = carbohydrates

FFA = free fatty acids
VLDL = very low density lipoproteins

OXIDATION OF FATS

As mentioned earlier, the natural fat is hydrolyzed to fatty acids and glycerol in the adipose tissue and liberated into the blood, the former transported as an albumin-FFA complex. The glycerol can be converted to α-glycerophosphate by the enzyme glycerokinase and later converted to dihydroxyacetone-phosphate. It can be utilized in the glycolytic pathway. The oxidation of fatty acids was studied by Knoop by tagging the methyl carbon with a phenyl group. He administered such tagged fatty acids to animals and analyzed the excretory product in urine. Whenever the original fatty acid had an even number of carbon atoms, he found the excretory product to be phenyl acetic acid. When the original fatty acid had an odd number of carbons, the excretory product was benzoic acid. From this Knoop (1905) proposed his 'β-oxidation theory' which has since been confirmed by isotopic and other techniques. According to this theory oxidation of fatty acid occurs at the β-carbon, resulting in the formation of a molecule of acetate from the terminal two carbons and leaving a residue of a fatty acid containing two carbons less than the original. The process repeats itself until the fatty acid with even number of carbons is completely converted to acetate molecules. The detailed steps are given below:

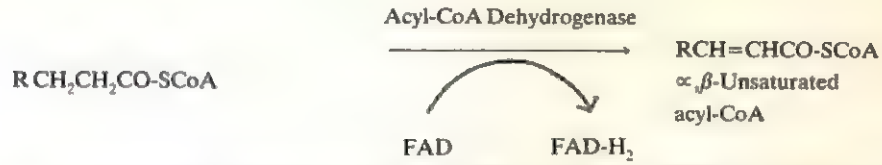
1. Activation of fatty acid:



The active fatty acid can now undergo oxidation.

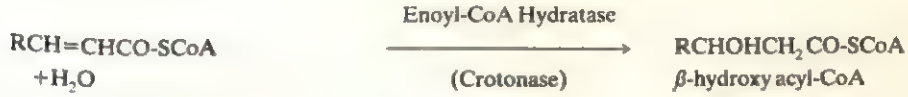
The enzymes which activate fatty acids by converting them to fatty acyl-CoA are called *'thiokinases'* or *'acyl-CoA synthetases'*. There are different enzymes to act on fatty acids of different chain lengths — i. c_2 and c_3, ii. c_4 to c_{12} and iii. c_{10} to c_{18} etc.

2. Formation of unsaturated acyl-CoA:

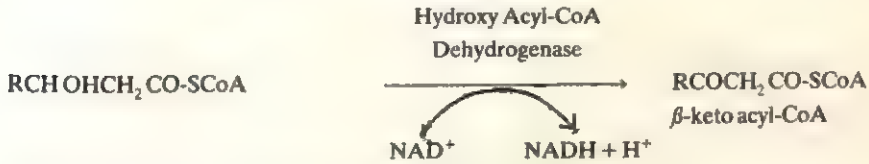


The hydrogen is taken up by FAD of the enzyme. Different enzymes are said to act on fatty acids of different chain lengths.

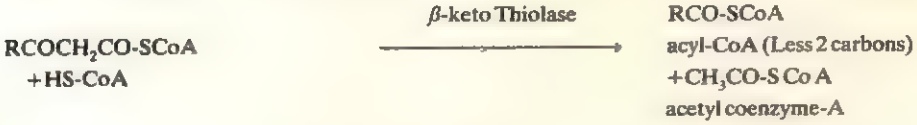
3. Formation of β-Hydroxy Acyl-CoA:



4. Formation of β-keto acyl-CoA:



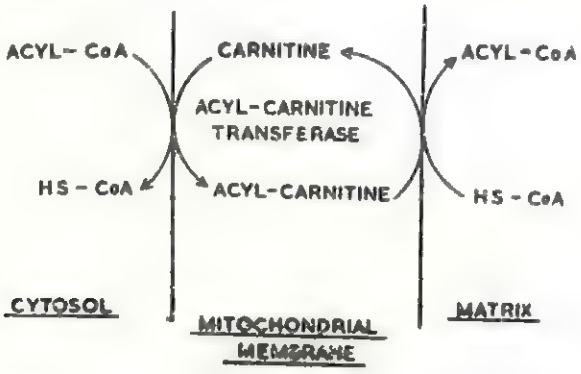
5. Thiolytic cleavage of acetyl-coenzyme A:



The acyl-CoA formed in this step is an active fatty acid containing two carbons less than the original and can undergo a similar set of reactions starting at reaction 2. This can repeat itself till a 4 carbon acyl-CoA (butyryl-CoA) is formed which is finally converted to 2 molecules of acetyl-coenzyme A.

All the enzymes required for β-oxidation are present in the mitochondria.

Fatty acyl-CoA is formed in the cytoplasm. This is impermeable to mitochondrial membrane. Acyl-CoA interacts with carnitine to form acylcarnitine which passes through the inner membrane. On the inner surface of the membrane, the acyl part is transferred to intramitochondrial CoA to form Acyl-CoA again and carnitine is released for further transport of acyl-CoA.

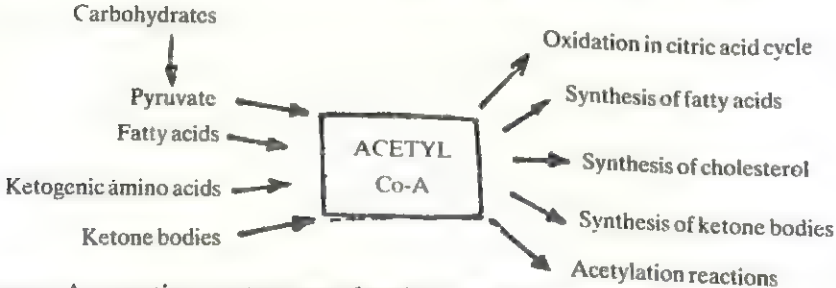


Carnitine is beta-hydroxy, gamma-trimethyl ammonium butyrate and has the following structure —

$(CH_3)_3N^+CH_2CH(OH)\,CH_2\,COOH$

The acetate (acetyl-coenzyme A) produced as a result of β-oxidation can enter the citric acid cycle and be oxidized in that cycle to carbon dioxide and water and yield energy. It may also take several other metabolic pathways as outlined below.

Sources and metabolism of active acetate (Acetyl-Coenzyme A)



Acetyl-coenzyme A or active acetate can thus be formed from carbohydrate, lipid as well as protein and can be oxidized in citric acid cycle to provide energy or it may be used for synthesis of fatty acids, cholesterol or ketone bodies. It can acetylate and form a number of biologically important substances — e.g.: acetyl choline, acetylated hexosamines and amino acids, and in the detoxication of sulfanilamide derivatives.

Energy available from oxidation: The oxidation of a long chain fatty acid will yield quite a large amount of energy. If palmitic acid is oxidized, it will result in 8 molecules of acetate and will run through the β-oxidation cycle 7 times. In each cycle of β-oxidation, energy can be obtained by reoxidation of the co-enzymes $FADH_2$ (step 2) and $NADH + H^+$ (step 4), the first yielding 2 ATP and the second 3 ATP. Thus a total of 5 ATP is realized when a fatty acid runs through the cycle once.

Since palmitic acid runs 7 times, energy by β-oxidation	.. 7x5=35 ATP
Each of the 8 molecules of acetate when oxidized in citric acid cycle will produce	.. 8x12=96 ATP
	Total = 131 ATP
In the initial activation step of fatty acid, one ATP is converted to AMP+PP resulting in loss of 2 energy rich phosphates	(—)2 ATP
	Hence net gain = 129 ATP

α-Oxidation of fatty acids: This is found to occur in the microsomal fraction of brain and other tissues and plants. This particularly involves the hydroxy fatty acids where the –OH is attached to the α-carbon.

ω-Oxidation: This is observed in the liver microsomal fraction. Medium chain length fatty acids are usually involved. First a –OH is added to the omega carbon which is then further oxidized to form an α ω-dicarboxylic acid. Now beta oxidation can occur from either end.

Oxidation of unsaturated fatty acids

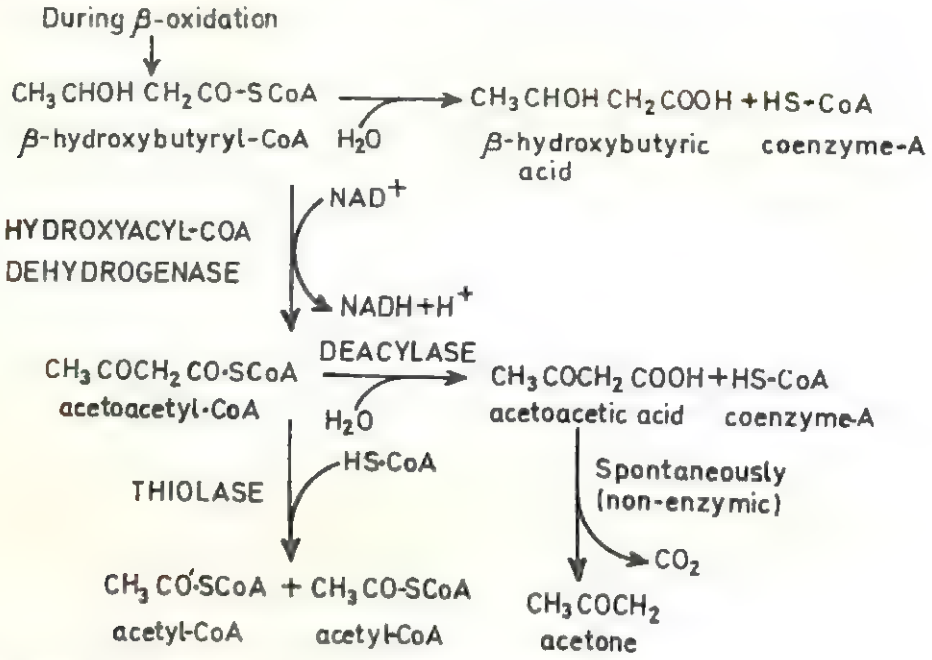
Beta oxidation proceeds as usual till the double bond comes to occupy a place between the second and the third carbon from the –COOH end (the product at this stage will be R.CH=CH.COSCoA). Since this is a product normally formed by the action of acyl-CoA dehydrogenase, the remaining enzymes continue their action to complete oxidation.

Microsomal peroxidation of polyunsaturated fatty acids

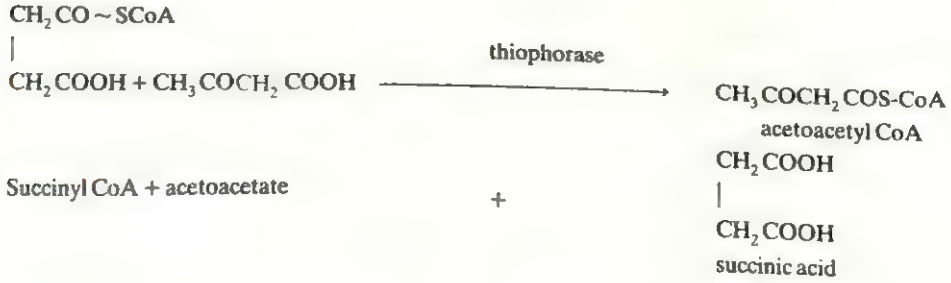
NADPH-dependent microsomal enzymes remove hydrogens and add oxygen at the double bonds to convert the polyunsaturated fatty acids to form corresponding hydroperoxides or endoperoxides. Vitamin E prevents the formation of these toxic peroxides by its autooxidant action.

Acetoacetic acid, β-hydroxybutyric acid and acetone are called the ketone bodies. They are readily interconvertible. Hydroxy butyryl-CoA and acetoacetyl-CoA are normally

Formation and Metabolism of ketone bodies:



produced in the penultimate steps of β-oxidation. Acetoacetyl-CoA may be also formed by condensation of 2 molecules of acetyl-coenzyme A. HMG-CoA is another source. A reversal of step 3 of cholesterol biosynthesis will produce a molecule each of acetoacetate and acetate. The enzyme acting in this direction is called 'HMG-CoA lyase.' Livers of fasting animals show a decrease in HMG-CoA reductase activity (HMG-CoA reductase is the enzyme that converts HMG-CoA to mevalonic acid, an important step in the synthesis of cholesterol). HMG-CoA is therefore acted upon by the cleavage enzyme resulting in the formation of acetoacetate. Of all tissues, liver has a very active deacylase enzyme acting on acetoacetyl-CoA. Hence acetoacetyl-CoA is rapidly converted to acetoacetic acid by the liver as it is formed. To utilize the acetoacetate, it has to be reactivated by acetoacetate thiokinase which adds coenzyme A or by transfer of coenzyme A from succinyl-CoA by an enzyme called thiophorase.

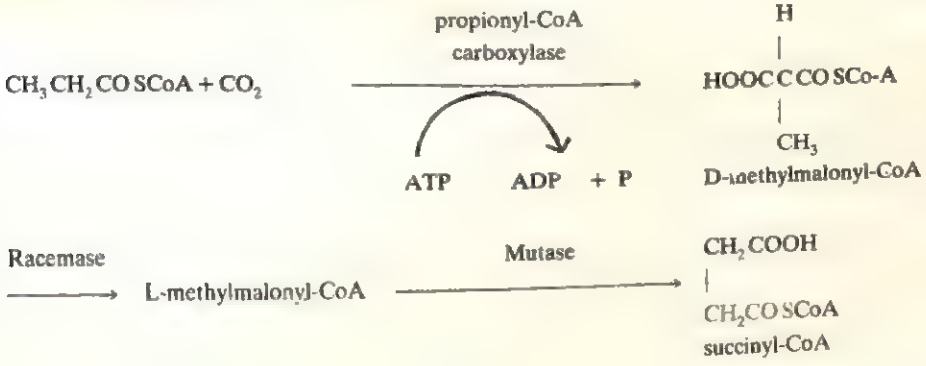


The acetoacetyl CoA is now broken into two molecules of acetyl CoA by thiolase (reaction 5 of beta-oxidation). The acetyl CoA can be metabolized in the usual way.

Both these enzyme activities are low in liver. Hence the acetoacetate produced by liver cannot be utilized by it but is let out into circulation. It is taken up by other tissues and promptly utilized, because, in these tissues, the reverse conditions are prevailing — a surplus of enzymes activating acetoacetate (thiokinase and thiophorase) and a deficit of deacylase. Thus ketone bodies are normally produced by liver and utilized by extrahepatic tissues. The blood levels of ketone bodies do not exceed 1 mg/100 ml and less than 1 mg are excreted in urine in 24 hours.

In conditions where increased amounts of fat are being metabolized e.g: diabetes mellitus and starvation, the liver produces these substances in large amounts beyond the ability of the tissues to take up and metabolize. This results in increased quantities of the ketone bodies in blood, and increased amounts are excreted in urine, conditions described as ketosis and ketonuria. Two of the ketone bodies — acetoacetic acid and β-hydroxy butyric acid — being acidic, their accumulation in blood lowers its pH and causes an acidosis.

Oxidation of fatty acids with odd number of carbon atoms: Though odd number of carbons are not present in natural fatty acids, there are mechanisms available in the organism for their metabolism also. After the usual repetitive steps of beta oxidation removing two carbons at a time, the ultimate product will be propionyl-CoA containing three carbons. This is further metabolized as follows:—



The succinyl-CoA can be metabolized in the citric acid cycle. The methylmalonyl-CoA mutase enzyme requires vitamin B_{12} as coenzyme. In the deficiency of this vitamin, excretion of methylmalonate and propionate in large amounts occurs in urine.

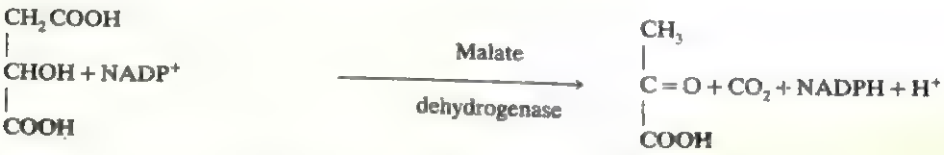
BIOSYNTHESIS OF LIPIDS.

Synthesis of fatty acids: Synthesis of fatty acids can occur in mitochondria as well as in cytoplasm. Synthesis in microsomes has also been reported.

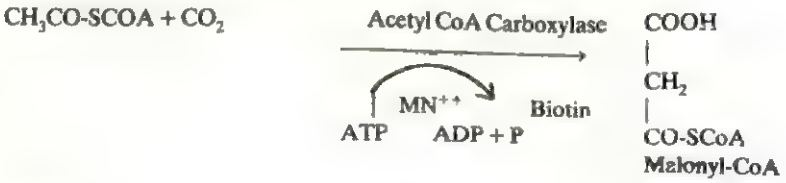
Mitochondrial system: The synthesis is mostly restricted to lengthening of an existing fatty acid by a reversal of the β-oxidation. Long chain fatty acids, stearic and palmitic, are synthesized. The coenzymes used in the reductive steps are both NADH + H^+ and NADPH + H^+. The enzymes of β-oxidation can all act in the reverse direction except the Acyl-CoA dehydrogenase (step 2). This is catalyzed by α, β-unsaturated acyl-CoA reductase (enoyl CoA reductase) and requires NADPH + H^+ as hydrogen donor. Pyridoxal phosphate is required in the initial step of condensation of acetyl-CoA with acyl CoA.

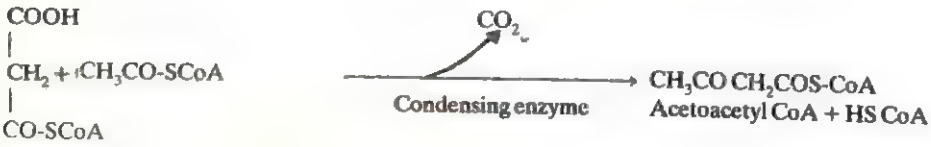
Extra mitochondrial system: This can synthesize fatty acids de-novo starting from acetyl-CoA. Acetyl-CoA is mostly formed in the mitochondria where pyruvate dehydrogenase is located. Mitochondrial membrane is impermeable to acetyl-CoA. Hence acetyl-CoA condenses with oxaloacetate to form citrate which now comes out of the mitochondria (permeable). Acetyl-CoA and oxaloacetate are released from this in the cytoplasm by the action of an enzyme called ATP-citrate lyase.

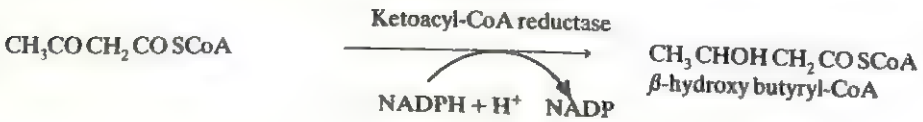
The NADPH + H^+ required in the biosynthesis is obtained partly by the oxidation of malate.



Palmitic acid is the fatty acid mainly synthesized. The steps are as follows:

1. 

2. 

3. 

4. $CH_3CHOCHCH_2CoSCoA \xrightarrow{\text{Hydratase}} CH_3CH=CHCO\text{-}SCoA + H_2O$

α,β-unsaturated butyryl CoA
(Crotonyl CoA)

5. $CH_3CH=CHCO\text{-}SCoA \xrightarrow[FMNH_2 \quad FMN]{\text{unsaturated acyl CoA Reductase}} CH_3CH_2CH_2CO\text{-}SCoA$ Butyryl CoA

$NADP^+ \quad NADPH + H^+$

The source of CO_2 for the first step is bicarbonate. In bateria, plants and lower forms of life, the individual enzymes are separate and a special protein called the 'Acyl Carrier Protein' (ACP) binds the acyl radicals. ACP is a single polypeptide chain of 77 amino acids. A serine moiety of this is in combination with phosphopantothene. Thus it is very similar to coenzyme-A.

In yeast, mammals and birds, the synthetase system exists as a multienzyme complex and ACP is a part of this complex. The fatty acid synthetase complex is a dimer, each monomer having a molecular weight of 250,000. The monomers are identical and contain ACP and seven enzyme components, arranged in the following order:—

Monomer 1	Monomer 2
1. ACP (contains 4 phospho pantothene residues)	Ketoacyl synthetase (contains –SH of cysteine)
2. Deacylase	Ketoacyl reductase
3. Malonyl transferase	Hydratase
4. Acetyl transferase	Unsaturated acyl reductase
5. Unsaturated acyl reductase	Acetyl transferase
6. Hydratase	Malonyl transferase
7. Ketoacyl reductase	Deacylase
8. Ketoacyl synthetase (contains -SH of cysteine)	ACP (contains 4 phospho pantothene residues)

Initially an acetyl-CoA combines with the cysteinyl –SH group of one of the monomers of the enzyme complex — say monomer 1. The reaction is catalyzed by acetyl transferase. Malonyl-CoA combines with the pantothenic –SH of ACP on monomer 2, catalyzed by the

enzyme, malonyl transferase. Acetyl-CoA from monomer 1 now interacts with malonyl-CoA on monomer 2 to form acetoacetyl-CoA and CO_2 is released. The acetoacetyl-CoA is formed on monomer 2. Subsequent reactions proceed on the same monomer 2 till the formation of butryl-CoA. Now, another molecule of malonyl-CoA is taken up by the pantothenil -SH of ACP of monomer 1 (which has taken up acetyl-CoA in the earlier sequence of reactions). The malonyl-CoA interacts with the butyryl-CoA, leading to the formation of a molecule of caproyl-CoA attached to the ACP of monomer 1.

The process is repeated, the pantothenil -SH and the ACP -SH of monomers 1 and 2 alternately taking up malonyl-CoA and acetyl-CoA till a chain length of 16 carbons is reached (palmityl-CoA). This is then hydrolyzed by the final enzyme 'deacylase' and is released into the cytosol.

In the biosynthesis of lipids, acetyl-CoA carboxylase is the rate limiting step. Long chain acyl-CoA molecules and glucagon inhibit the enzyme and thus cause inhibition of fatty acid synthesis. This is an example of feed-back inhibition. The enzyme also decreases in the fasting state, in diabetes mellitus and in conditions of excessive dietary fat.

Palmitic acid formed above is further lengthened to form stearic and arachidonic acid derivatives in the endoplasmic reticulum.

Unsaturated fatty acids: They are not synthesized de novo, but are formed by desaturation of the saturated fatty acids. The enzyme system for this is also located in the endoplasmic reticulum. Animal tissues lack the enzymes that can introduce double bonds between the seven carbons from the CH_3 end of the fatty acid. Linoleic, linolenic and arachidonic acids belong to this group. Hence they cannot be synthesized by higher animals and have to be supplied in diet containing fats from vegetable sources.

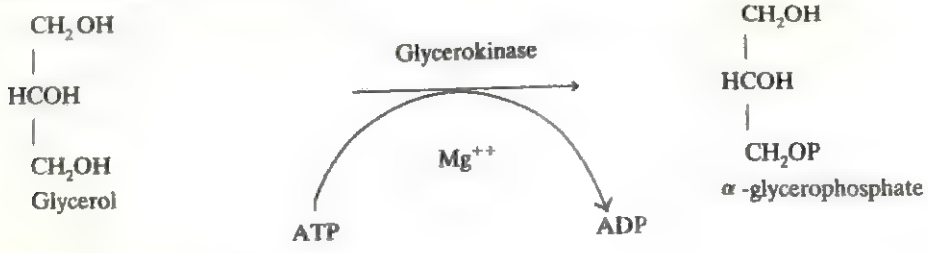
Desaturation and elongation reactions occur more extensively in liver than other tissues.

Microsomal system: There is also a system present in microsomes which can lengthen existing fatty acid chains. This also uses malonyl-CoA as acetyl donor and NADPH + H^+ as reducing coenzyme.

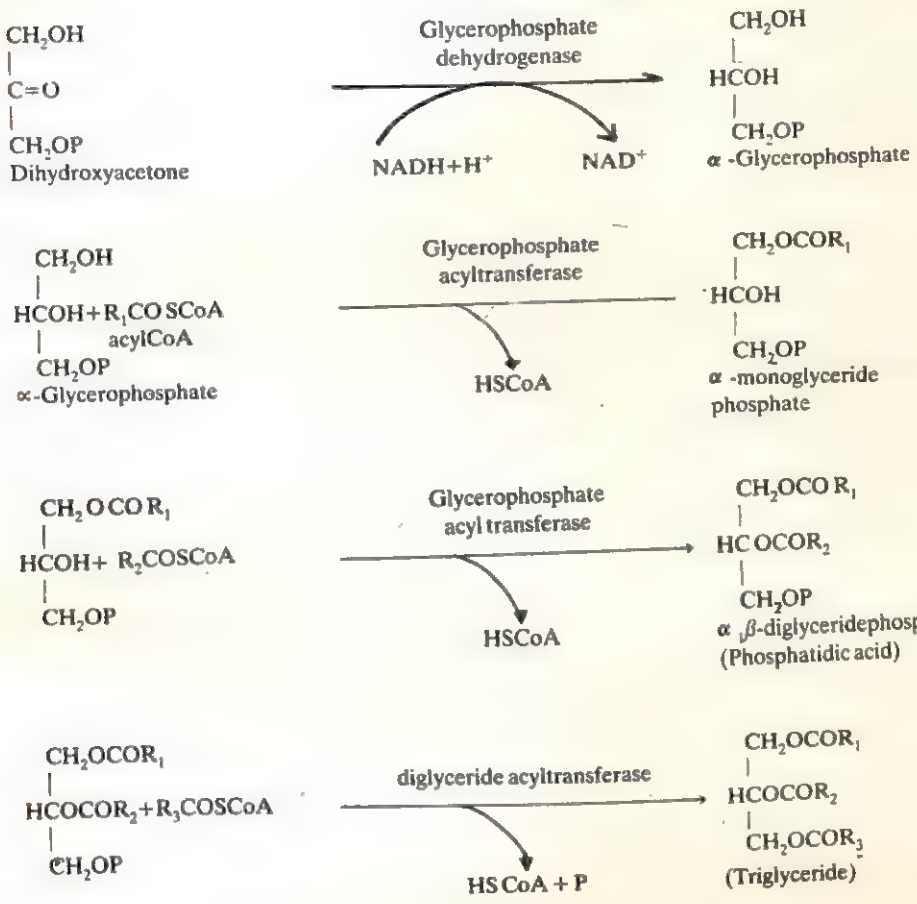
The NADPH + H^+ required as coenzyme in all the three systems is formed in the HMP pathway of glucose metabolism. The two pathways go together in most tissues.

Carnitine (see under beta oxidation) can not only help in the transport of fatty acids from cytoplasm into mitochondria but also in the reverse direction.

Synthesis of triglycerides: The fatty acid can be incorporated after activation to acyl-CoA. Glycerol also has to be activated.



The glycerol can be derived also from dihydroxyacetone phosphate from glucose obtained during glycolysis.



The enzymes are called glycerophosphate acyltransferases. The synthesis is mainly microsomal and to a lesser extent can also occur in mitochondria.

Synthesis of phospholipids: The steps are same upto formation of phospnatidic acid ($\alpha\beta$-diglyceride phosphate). This combines with cytidine diphosphate of CTP to form CDP-diglyceride. The CDP-diglyceride can now interact with inositol (or active choline or active ethanolamine) to form inositol phosphatide or lecithin or ethanolamine phosphatide and CDP is released. Activation of choline and ethanolamine is brought about by their combining with CTP to form CDP-choline or CDP-ethanolamine after preliminary activation by ATP. The phosphatidyl ethanolamine can interact with serine to form phosphatidyl serine and ethanolamine.

Breakdown of phospholipids: Several enzymes called 'phospholipases' are capable of hydrolyzing the fatty acids from phospholipids.

Sphingomyelins: Sphingosine, the alcohol moiety, is formed from palmitic acid and serine and requires coenzyme A and pyridoxal phosphate. The sphingosine interacts with CDP-choline and then the amino group is acylated to form sphingomyelin.

Cerebrosides: Sphingosine can interact with UDP-galactose and amino group can be acylated to form cerebroside.

Functions of phospholipids:

1. They are important constituents of the lipoproteins of the cell wall, mitochondrial membrane and other intracellular membranes. They seem to regulate the permeability and other physico-chemical properties of the membranes. Some of the enzyme activities such as those of the electron transport chain of the mitochondrial membrane are lost if the phospholipids are removed from the membrane.

2. Phosphatidylserine and phosphatidylethanolamine play important role in the blood clotting mechanism.

3. The phospholipids help in the digestion and absorption of lipids by aiding emulsification of lipids in the intestine and by forming the lipoprotein layer around the chylomicrons.

4. Plasma lipoproteins are rich in phospholipids and serve the function of transport of lipids.

5. Phosphoinositides and phosphatidic acids function in the transport of inorganic ions, mainly cations, across membranes.

6. Phospholipid formation in the liver seems to be an essential prerequisite for the rapid oxidation of fatty acids in that viscera.

7. Phospholipids act as surfactants in the lung and help keep the alveoli their shape and function. Phospholipids start forming on the alveolar membrane about the 30th week of gestation and reach physiological levels by 35th week. Premature infants born before the 35th week develop respiratory distress and cyanosis.

The maturity and the viability of the fetus can be assessed by an analysis of the amniotic fluid for its phospholipid content. Lecithin: sphingomyelin ratio is low at 30 weeks and rises to 2:1 by the 35th week in the amniotic fluid.

Essential Fatty Acids:

These are polyunsaturated (polyenoic) fatty acids having two or more double bonds. They cannot be synthesized by the mammalian tissues, because the tissues do not have the ability to introduce a double bond to link the terminal seven carbons from the methyl end. (In a 18 carbon fatty acid of the stearic acid series the terminal seven carbons from the methyl end

will be from C-12 to C-18; in the 20 carbon arachidic acid series, they will be C-14 to C-20). But some of these polyunsaturated acids are structural components of mammalian tissues and have therefore to be supplied preformed in food. They are hence called 'essential fatty acids'. They are–

1. C-18 acids (derivatives of stearic acid).

   (i) linoleic acid: 2 double bonds between C-9 and C-10 and C-12 and C-13.

   (ii) Linolenic acid: 3 double bonds between C-9 and C-10, C-12 and C-13 and C-15 and C-16.

2. C-20 acid (derivative of arachidic acid):

   (iii) arachidonic acid: 4 double bonds between
   C-5 and C-6, C-8 and C-9,
   C-11 and C-12 and C-14 and C-15.

(However, mammalain tissues can convert linoleic acid to linolenic and arachidonic acids, provided pyridoxine is also available. Hence linoleic is the only fatty acid which is absolutely indispensable).

Their deficiency in experimental animals causes a decrease in growth rate with inability to reproduce. Skin shows characteristic scaly lesions, a necrosis of tail and a hemorrhagic necrosis of the kidney.

The capillary resistance is decreased and permeability increased in such animals.

Human infants on low fat diets show rough, desquamating lesions, particularly in the diaper region, or eczematous skin lesions on other parts of the body. These are relieved by adding adequate amounts of fat to the diet. Similarly, for normal rate of growth, maturation, pregnancy and lactation, optimal amount of fat in diet is required. The beneficial effects of fat in all these instances are probably on account of their content of essential fatty acids. The essential fatty acids are also found to exert a protective influence against the harmful effects of exposure to X-rays.

The essential fatty acids are present in the structural lipids of the cell and are important constituents of mitochondrial lipids. They are also concentrated in the testis and ovary. Phospholipids contain good amounts of these acids. They are also used for the esterification of cholesterol. Their deficiency can cause fatty liver and high cholesterol levels in blood.

The C-20 fatty acids of the arachidonic type can be converted by the mammalian tissues to pharmacologically active compounds called 'prostaglandins'.

CHOLESTEROL METABOLISM.

Average diet supplies about 0.3 gram of cholesterol a day. But over 1 gm cholesterol is synthesized in the body. Hence endogenous synthesis is far more than dietary supply. The complex ring structure is shown to be synthesized from acetate (Bloch and Rittenberg, 1942).

15 of the 27 carbons coming from methyl group and the remaining 12 from carboxylic group of acetate. The liver and the intestine are the principal sources for blood cholesterol and synthesize about 1 gm. each day. The skin, adrenal glands, testis and ovary also synthesize small amounts of the substance mainly for the formation of specialized substances (eg: vitamin D_2 and sebum from skin; steroid hormones from others). They do not contribute to plasma cholesterol. Adipose tissue, aorta, muscle and brain also synthesize small amounts. The synthesis occurs mainly in the microsomal and soluble fractions of the cell.

K. Bloch, F. Lynen, G. Popjak and J. Cornforth elucidated the steps in the biosynthesis of cholesterol.

1. *Formation of acetyl-CoA:* A molecule of acetic acid is activated by combining with coenzyme A. The reaction requires energy from the breakdown of ATP and is catalyzed by the enzyme acety-CoA synthetase. Magnesium ions are also required.

2. *Formation of acetoacetyl-CoA:* Two molecules of acetyl-CoA then condense to form an acetoacetyl-CoA molecule.

3. *Formation of HMG-CoA:* The acetoacetyl-CoA now condenses with one more molecule of acetyl-CoA to form beta-hydroxy, beta-methyl glutaryl-CoA (abbreviated as HMG-CoA). The enzyme which mediates this reaction is called HMG-CoA synthetase.

4. *Formation of mevalonic acid:* The HMG-CoA is reduced by an $NADPH+H^+$ depending reductase to form mevalonic acid.

The HMG-CoA reductase enzyme exists in an active, dephosphorylated form and an inactive, phosphorylated form and is subjected to hormonal influence through the cascade system of enzymes as in the case of glycogen synthase and phosphorylase. Epinephrine and glucagon favour phosphorylation and inactivation of the enzyme and thus inhibit cholesterol biosynthesis. Insulin has an opposite effect and stimulates cholesterol biosynthesis.

5. *Formation of isopentenyl pyrophosphate:* In a series of 3 steps involving phosphorylation by 3 ATP and loss of one of the 6 carbons of mevalonic acid as CO_2 a five carbon compound, isopentenyl pyrophosphate, is formed (also known as the isoprenoid unit). The isoprene units can exist in more than one isomeric form.

6. Two isoprene units condense to form a geranyl pyrophosphate (10 carbons).

7. One more isoprene unit condenses with geranyl pyrophosphate to form farnesyl pyrophosphate (15 carbons).

8. Two farnesyl units combine to form squalene (30 carbons).

Other polyisoprenoids like *dolichol and ubiquinone* are also synthesized from farnesyl pyrophosphate. Dolichol is a long chain alcohol (C_{95}) and takes part in glycoprotein synthesis by transferring carbohydrate residues to aspargine residues of the polypeptide.

9. Closure of the ring occurs to form lanosterol.

10. Lanosterol is then converted through 14-desmethyl lanosterol, zymosterol, Δ 7, 24-cholastadienol, 24-dehydrocholesterol (desmosterol) and finally to cholesterol. All these steps are shown in fig. 15-2.

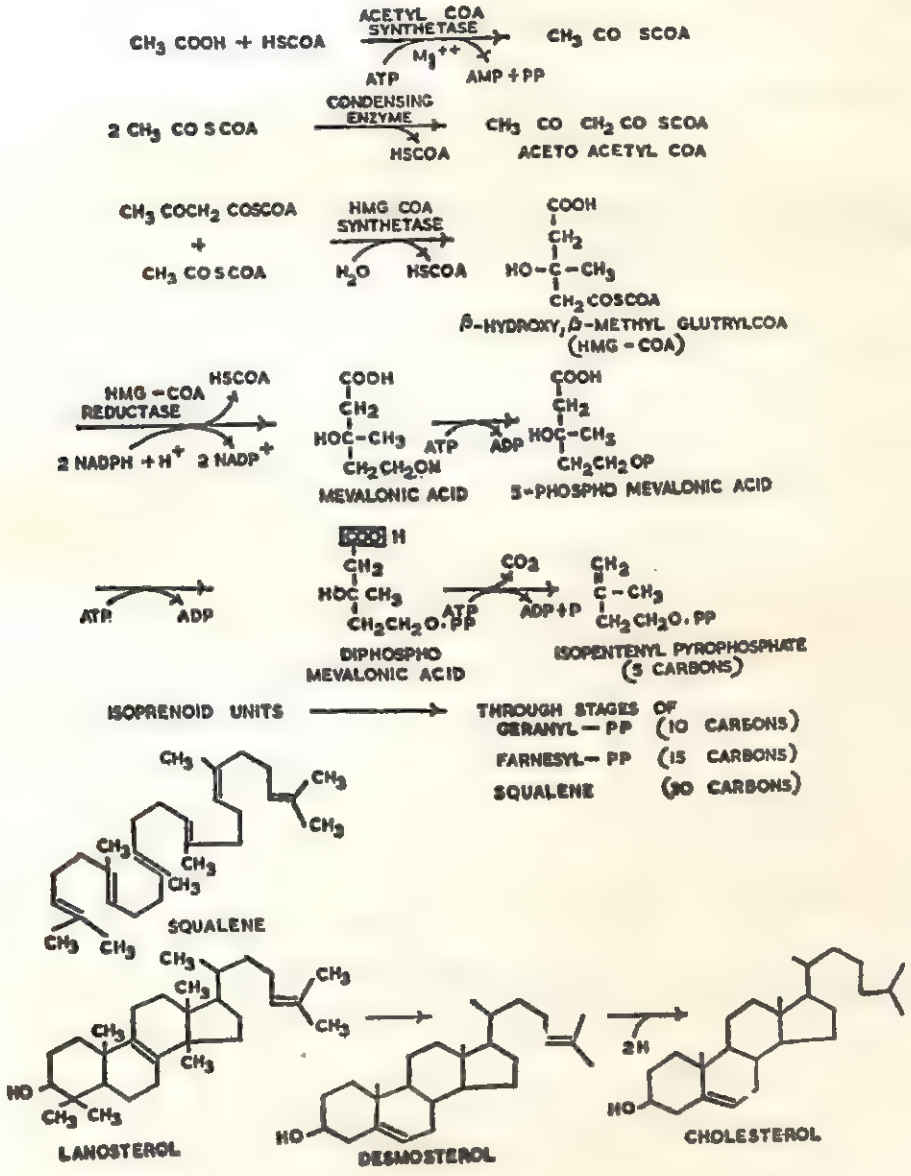


Fig. 15-2. Biosynthesis of cholesterol.

The reductive reactions in the cholesterol synthesis require $NADP.H+H^+$ and they are dependent on its production through HMP pathway of glucose metabolism.

After the formation of squalene, subsequent steps appear to take place on a special carrier protein called 'squalene and sterol carrier protein.' The synthesis of steroid hormones, bile acids etc., also seem to occur in association with the protein. The feed back inhibition of HMG-CoA reductase is also probably mediated by this sterol-carrier protein complex.

A second site of regulation of cholesterol biosynthesis is in the cyclization of squalene to form lanosterol.

The complex set of reactions above can be completed and labelled acetate is incorporated into cholesterol by liver in the matter of a few minutes.

Blood Cholesterol: Dietary cholesterol and also the cholesterol synthesized in the intestinal mucosa are esterified in the intestinal mucosa and along with other lipids incorporated into VLDL and chylomicrons. These are absorbed into the blood stream. Liver also synthesizes cholesterol and secretes into the blood stream along with triglycerides as VLDL. The triglycerides are removed from VLDL by peripheral tissues, mainly adipose tissue, by the action of lipoprotein lipase. The VLDL, losing much of the triglycerides, is transformed to LDL, which contains a large amount of cholesterol and its esters. Different tissues including the liver take up LDL at specific LDL receptors which are present on their cell surfaces. The LDL are then transported into the cell. The apoprotein (apo-B mainly) is hydrolyzed to its constituent amino acids. Cholesterol ester is broken down to free cholesterol and fatty acid by lysosomal enzymes. Some cholesterol is incorporated into the cell membranes and the rest re-enters the plasma, where it is taken up by HDL and returned to the liver. The free cholesterol taken up by the HDL is esterified to cholesterol ester by transfer of a fatty acid from lecithin (the fatty acid esterifying the beta carbon of choline) by an enzyme *'lecithin: cholesterol acyltransferase (LCAT)'*. A deficiency of this enzyme in some individuals causes high levels of free cholesterol in plasma. The enzyme is mainly synthesized in the liver. Hence, deficiency of the enzyme can also occur in parenchymal liver disease and can cause an increase in the free: ester cholesterol ratio.

A healthy adult male has plasma cholesterol of about 200 mg/100 ml. Of this, about 150 mg is esterified and 50 mg is in the free form.

Excretion: 80% of cholesterol is converted to bile acids — mainly cholic acid and chenodeoxycholic acid — in the liver. They are activated to corresponding cholyl-CoA and then combine with glycine or taurine to form glycocholic acid or taurocholic acid. The sodium and potassium salts of these bile acids are called the bile salts. Bile salts are excreted in the bile and serve important functions in the absorption of lipids by the intestinal mucosa. Along with the lipids, most of the bile salts are also reabsorbed. About 500 mg are lost in the feces daily.

Due to bacterial action, some of the cholic acid and chenodeoxycholic acid is converted to deoxycholate and lithocholate. They also may be reabsorbed from the lower illeum and are called *'secondary bile acids'*.

Some cholesterol is excreted as such through bile. This is reduced by intestinal bacteria and is excreted in feces as *'coprostanol'*. Such of the dietary sterols which are not absorbed, mainly the plant sterols, are also excreted in feces.

Cholesterol and atherosclerosis: Atherosclerosis is a major cause of death in affluent nations and the richer sections of even poorer nations. The disease is characterized by accumulation of cholesterol and other lipids under the tunica intima leading eventually to a breakdown of the endothelial lining. This leads to narrowing of the arteries, clotting of blood over the ulcerated area (thrombosis) and dislodgment of the clot which may obstruct the coronary blood vessels or cerebral blood vessels (embolism). Instant death may occur or the subject may recover with possibility of relapse any time. Increased plasma cholesterol level is said to predispose to the development of this disease. Increase in cholesterol level is always associated with increase in β-lipoprotein (Sf 10-400) level in plasma. Pyridoxine deficiency favours the development of atherosclerosis in the rhesus monkey. Excessive cigarette smoking is another important contributing factor. In most cases, there is also a hereditary predisposition to atherosclerosis.

Efforts to decrease chlolesterol levels are made to prevent and also to treat the disease. Ingestion of fats rich in polyunsaturated fatty acids is found to lower blood cholesterol level. Thus vegetable oils such as corn oil, safflower oil, gingely or groundnut oil are good in keeping the cholesterol levels low. Animal fats like butter, ghee and curd tend to raise plasma cholesterol. The one exception in vegetable fats is coconut oil which tends to raise blood cholesterol. This is because coconut oil is poor in polyunsaturated fatty acids. Cholesterol esterified with polyunsaturated fatty acids seems to be more easily transported into the tissues and more readily metabolized. Several drugs are used to decrease the absorption of cholesterol or to decrease its synthesis by liver or to prevent reabsorption of bile salts.

Clofibrate (Atromid): Decreases biosynthesis of cholesterol in liver and causes excretion of neutral sterols in feces. It also lowers the plasma triglycerides and VLDL.

Nicotinic acid: High doses cause a lowering of plasma VLDL levels. Nicotinamide has no such effect.

Heparin: Enhances the activity of lipoprotein lipase and lowers the triglyceride levels of chylomicrons and VLDL. Cholesterol levels are also lowered.

Cholestyramine: It is an anion-exchange resin. It has strong affinity for bile acids and causes their excretion in large amounts. Plasma LDL and cholesterol levels are both decreased.

Thyroid hormone analogues: D-Thyroxine and some other analogues lower the plasma cholesterol and LDL levels with little or no effect on the basal metabolic rate.

Estrogens: Young females with high estrogen levels show lower cholesterol levels than men of similar age. But on account of side effects like gynaecomastia, loss of libido and impotence, their use is of little practical value. They also increase VLDL levels due to rise in plasma triglycerides.

ABNORMALITIES IN LIPID METABOLISM

1. Obesity: Excessive deposition of fat in the depots with decreased mobilization causes obesity. Obesity of non-endocrinal causes is always due to ingestion of more food than necessary to meet the metabolic needs of the adult. Castration, hypothyroidism and hyperinsulinism are some of the endocrine causes. Lesions of hypothalamus increase appetite and cause obesity.

2. Cachexia: It is the opposite condition where the fat depots are very scanty. The causes are 1. toxic — due to conditions like carcinoma, prolonged illness like tuberculosis and malnutrition and 2. endocrine disorders — hyperthyroidism, severe diabetes and hypopituitarism.

3. Idiopathic hyperlipemia: This is an inborn error associated with high lipid levels of plasma. The fasting plasma is milk like and contains particularly high levels of triglyceride and fatty acids. Cholesterol and phospholipids also may be increased.

4. Gaucher's disease: Cerebrosides are increased in brain, liver and spleen. There is an imbalance between the synthesis and the breakdown of this lipid.

5. Niemann-Pick's disease: Sphingomyelins accumulate in liver, spleen, bone marrow, lung and lymph nodes. Gangliosides of brain show degeneration. Several variants of the disease occur. The abnormality here also is an imabalance between synthesis and breakdown of the sphingomyelins.

6. Tay-Sachs Disease: Abnormal glycosides accumulate in brain. There is retarded development, paralysis, dementia and blindness. The child does not usually survive beyond 2 to 4 years.

7. Fabry's Disease: An abnormal galactosyl-sphingolipid accumulates in many tissues. Death occurs due to cardiac or renal failure.

8. Hyper and Hypolipoproteinemias: Inborn errors of metabolism leading to an increase or decrease or absence of the lipoprotein components of plasma occur. The fraction most often involved is the β-lipoprotein.

A. Hypolipoproteinemias:

1. Abetalipoproteinemia: It is an inherited disease in which apo-B and betalipoprotein (LDL) are absent in the plasma. Other lipids including triglycerides and chylomicrons are low. Plasma cholesterol is low. Triglycerides accumulate in the liver and the intestinal mucosa.

2. Hypobetalipoproteinemia: The beta lipoprotein fraction (LDL) is less than one half of normal.

3. Familial alfalipoprotein deficiency (Tangier's Disease): Plasma HDL and apo-A are absent. Cholesterol esters tend to accumulate in tissues. Chylomicrons are produced normally and there is *no* accumulation of triglycerides in liver.

B. Hyperlipoproteinemias:

Type I: Chylomicrons are cleared slowly from plasma, resulting in high chylomicron levels in plasma. There is a deficiency of lipoprotein lipase (clearing factor). The condition can be controlled by a low fat diet.

Type II: Familial hypercholesterolemia: There is an increase of LDL and cholesterol in plasma. Either the LDL binding to the cell membrane is absent or poor, or the transfer of the bound LDL to the cytosol is defective. Normally, intra-cellular cholesterol levels act as a feed-back inhibitor to HMG-CoA reductase. The mechanism fails in this conditon. Hence, there is excessive synthesis of cholesterol by the liver leading to hypercholesterolemia and high plasma LDL. The LDL receptors on the cell membrane are much diminished in number or may even be absent altogether.

Cholesterol storage disease (Wolman's disease): In this condition, the liposomal lipase which hydrolyzes cholesterol esters taken up by the cell is deficient. Hence, cytoplasm accumulates cholesterol esters.

Type III: Both LDL and VLDL are increased. Cholesterol and triglycerides are increased in the plasma.

Type IV: There is excessive endogenous synthesis of triglycerides leading to high levels of plasma triglycerides and VLDL. There is a corresponding increase of cholesterol also. Glucose tolerance is commonly decreased and the affected individuals tend to be obese. LDL and HDL levels are low.

Type V: chylomicrons and VLDL are increased in plasma. LDL and HDL are decreased. As in type IV, carbohydrate tolerance may be decreased and affected individuals may be obese.

Of the above hyperlipoproteinemias, type II is most common. Type IV is also fairly common. Types I, III and V are rare.

The data are summarized in Table 15-2.

TABLE 15-2

Hyperlipoproteinemias

Type	Lipoprotein abnormality	Plasma levels of cholesterol	Plasma levels of triglycerides	Associated factors
I	Chylomicrons increased	Increased	Increased	Occurrence rare. Lipoprotein lipase is deficient.
II	LDL increased	Increased	Increased or normal	Occurrence common. Associated with xanthomata and ischemic heart disease.
III	LDL and VLDL increased	Increased	Increased	Rare occurrence. Xanthomata present.
IV	VLDL increased	Increased or normal.	Increased	Excessive synthesis of lipid from carbohydrate. Occurrence common.
V	VLDL and chylomicrons increased	Increased	Increased	Uncommon occurrence. Associated with ketotic diabetes.

References:

Ansell G.B. and Hawthorne, J.N.: Phospholipids, Chemistry, Metabolism and Function, Elesevier, N.Y. 1964.
Bloch, K. (ed): Lipid Metabolism, Wiley, N.Y., 1960.
Bloor, W.R.: Biochemistry of the Fatty Acids and Their Compounds, the Lipids. Reinhold, N.Y., 1943.
Cook, R.P. (ed): Cholesterol, Its Chemistry, Biochemistry and Pathology. Acad. Pr., N.Y., 1958.
Dawson, R.M.C. and Rhodes, D.N. (eds): Metabolism and Physiological Significance of Lipids Wiley, N.Y., 1964.
Deuel, H.J.Jr: The Lipids, Their Chemistry and Biochemistry, vols. 1 to 3. Interscience N.Y., 1951, 55 and 57.
Feiser, L.F. and Feiser, M: Steroids, Reinhold, N.Y. 1959.
Graw, F.C. (ed): Cellular Compartmentation and Control of Fatty Acid Metabolism, vol. 4, Acad. Pr., N.Y., 1968.
Hsai, D.Y.: Inborn Errors of Metabolism, Part I.
2nd ed., Yearbook Medical, Chicago, 1966.
Jeanrenaud, B. and Hepp, D. (eds): Adipose Tissue Regulation and Metabolic Functions. Acad. Pr., N.Y. 1970.
Katz, L.N,. Nutrition and Atherosclerosis.
Lea and Febiger, Philadelphia, 1958.

Kinsell, L.W.: (ed): Adipose Tissue as an Organ.
Thomas, Springfield, Ill. 1953.

Kritchevsky, D: Cholesterol, Wiley, N.Y., 1958.

Masoro, E.J.: Physiological Chemistry of Lipids in Mammals.
Saunders, Philadelphia, 1968.

Meng, H.C. (ed): Lipid Transport, Thomas, Springfield, Ill, 1964.

Page, I.H. (ed): Chemistry of Lipids as related to Atherosclerosis.
Thomas, Springfield, Ill: 1958.

Popjak G. and Grant, J.K. (eds): The Control of Lipid Metabolism.
Acad. Pr., N.Y., 1963.

Reynold, A.E. and Cahill, G.F. Jr. (eds): Adipose Tissue.
Williams & Wilkins, Baltimore, 1965.

Rodahl, K. and Issekutz, B. (eds): Fat as a Tissue.
McGraw-Hill, N.Y., 1964.

Searcy, R.L. and Bergquist, L.M. Lipoprotein Chemistry in Health and Disease.
Thomas, Springfield, III 1962.

Sinclair, H.M. (ed): The Essential Fatty Acids.
Acad. Pr., N.Y., 1958.

Stanbury, J.B., Wyngaarden, J.B. and Fredrickson, D.S. (eds):
The Metabolic Basis of Inherited Disease, 2nd ed., McGraw-Hill, N.Y., 1966.

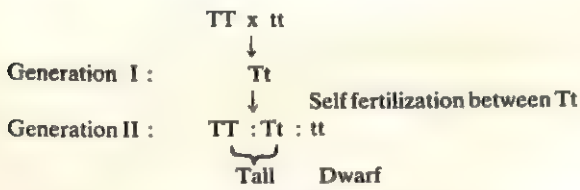
Wakil, S. (ed): Lipid Metabolism, Acad. Pr., N.Y., 1970.

16

GENETICS AND NUCLEIC ACIDS

GENETICS

George Mendel enunciated the first principles in genetics in 1865 based on his experiments with Pisum sativum (green pea). He crossed tall plants (T) with dwarfed plants (t) and from his observations on the successive generations of plants came to the conclusion that there are two distinct genes in the green pea — one for the tall characteristic and one for the dwarf characteristic.



TT, the types of genes inherited denotes the 'genotype'. Tall and dwarf, the actual character observed in the progeny indicates the 'phenotype'.

The gene T is said to be dominant (since in its presence tallness manifested in spite of the simultaneous presence of gene t), and the gene t is said to be recessive. Mendel studied several such isolated characters as well as combinations of characters.

Chromatin

Chromsomal material extracted from cell nuclei of eukaryotic cells is called chromatin. It contains equal amounts of DNA and proteins. The proteins are mainly histones (basic) and a small amount of acidic non-histone proteins. Small amounts of RNA are also present. Electron microscopy shows spherical particles called '*nucleosomes*' connected by DNA filaments.

The histones are a heterogeneous group and consist of several entities named Hl, H2A, H2B, H3 and H4. Hl histones are somewhat loosely bound to chromatin and can be removed by salt solutions. The rest of the chromatin now becomes soluble. When the proteins are freed from chromatin, they tend to associate themselves in a distinct pattern. H3 and H4 aggregate in pairs to form a tetramer ($H3_2$ - $H4_2$) and H2A and H2B to form dimers (H2A-H2B). Thus Hl, $H3_2$-$H4_2$ and H2A-H2B form three distinct groupings. When added to DNA in the correct proportion, the nucleosome can be reformed. The DNA winds in a left handed helical pattern over an octamer consisting of one $H3_2$-$H4_2$ tetramer and two H2A-H2B dimers. Hl only helps to seal these components together. An acidic nuclear protein called '*nucleoplasmin*' is required for the assembly of the histones and DNA to form the nucleosome. An enzyme called *DNA topoisomerase I* is also required.

The nucleosomes arranged side by side constitute a chromatin fibril which has a diameter of 10nm. On the other hand, the fibre may coil itself in such a way that there are 6 to 7 nucleosomes to a turn to form a thicker strand of 30nm diameter. Hl histone stabilizes this 30nm structure.

Active chromatin:

Though every cell contains genetic information for all the proteins capable of being synthesized by any cell, only some proteins are synthesized by particular cells. Thus, the genetic information common to all cells is expressed differently in different cells. Some genes are said to be active in each cell while others remain inactive. In the active genes, the nucleosome structure is either altered or altogether absent, thus exposing the DNA. These regions of DNA are characteristically free of 5-methyl deoxycytidine. The Hl histone is replaced by non-histone proteins. Such active chromatin regions are sensitive to DNAse I action, while the rest of chromatin is not. There is a portion of single stranded DNA upstream of the active gene which is even more sensitive to DNAse I.

The densely packed inactive chromatin is called *'heterochromatin'* and the lightly packed active chromatin is called *'euchromatin'*. The former stains densely with nuclear stains, while the latter stains less densely.

Chromosomes

During metaphase, chromosomes consist of two densely packed identical sister chromatids linked together at a region called the *centromere*. Each sister chromatid is a double stranded DNA Molecule. During interphase, the packing becomes less dense.

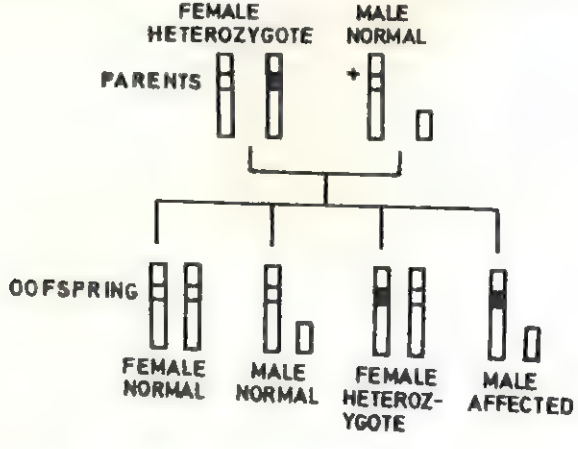
Chromosome: 1. centromere
2. chromatids

Genetic organization

In the diploid human cells, the genome consists of 7×10^9 base pairs of DNA distributed in 23 pairs of chromosomes. This is sufficient for about 3 million pairs of genes. However, not more than 100 thousand proteins are synthesized in the human tissues. Hence most of the DNA is noncoding for protein synthesis and serves other functions like regulation of gene expression, and gene function. The heterogenous nuclear RNA (hnRNA) translated from a coding portion of DNA will contain several noncoding sequences of RNA corresponding to the noncoding regions of the DNA. The noncoding regions are called the *'INTRONS'* and the coding regions are called the *'EXONS'*. This type of an arrangement whereby functional portions of the DNA alternate with non-functional portions makes it easy to repair the genes, if necessary, and for splicing and rearranging the genes when necessary by breaking the DNA molecule at its non-functional region.

Causes of Mutation: The exact causes are not known. Environmental temperature, atmospheric oxygen, nutrition, exposure to X-rays, ultraviolet rays and a host of other causes are incriminated. If the mutation is severe and life-endangering, the offspring do not survive and the mutant gene is therefore eliminated without further propagation. If it is not that severe, then it gets propagated through subsequent generations. Consanguinity (marriages between close relations — say first cousins) increases the chances of producing the homozygous state and the appearance of full-fledged metabolic abnormality in the offspring.

Sex-linked inheritance: Of the 23 pairs of chromosomes, the male cell has one unequal pair called the XY pair. The X chromosome is long while the Y chromosome is short. In the female, both are X chromosomes and are long. This pair of chromosomes (XY or XX) are called the sex-chromosomes. The remaining 22 pairs are called autosomes. The gene for hemophilia is carried on the upper portion of the X chromosome. If mating occurs between a heterozygous female and a normal male, the offspring will inherit the genes as follows:-



Of the two male children, one will show the disease. The other is normal. Of the two female children, one is heterozygous and the other normal.

Detailed studies have been made on all the 23 pairs of chromosomes. They can be classified into 7 groups and can be identified individually.

METABOLISM AND BIOSYNTHESIS OF NUCLEIC ACIDS

The protein moiety of the nucleoproteins present in food is acted upon by the proteolytic enzymes of the gastric and intestinal juices and the amino acids are absorbed and metabolized as usual. The nucleic acids are acted upon by nucleases (ribonuclease and deoxyribonuclease) of the pancreatic and intestinal juices to produce mononucleotides. Phosphatases (nucleotidases) from the intestinal secretion hydrolyze the nucleotides to form nucleosides and inorganic phosphate. The nucleosides are absorbed by the intestinal mucosa and carried through portal blood to liver and from there through systemic circulation to other viscera. They contain the enzymes nucleosidases which liberate the free purine or pyrimidine base and ribose or deoxyribose from the nucleotides. The digestion and absorption are summarized below.

Nucleoprotein
↓ Proteolytic enzymes of gastric and pancreatic juice
Nucleic acid
↓ RNA-se and DNA-se of pancreatic and intestinal secretions
Nucleotide
↓ Nucleotidase (intestinal phosphatase)
Nucleoside + Phosphate
Tissue nucleosidase | (Absorbed and taken to liver, kidney, spleen, bone marrow etc.)
↓
Purine or Pyrimi dine base
+
Ribose or Deoxyribose

The majority of dietary purines and pyrimidines are catabolized to uric acid and other products. Practically none is incorporated into tissue nucleic acids. However, parenterally administered nucleosides and nucleotides are incorporated as such into tissue nucleic acids (e.g. thymidine into DNA).

BIOSYNTHESIS OF NUCLEIC ACIDS

Before considering the biosynthesis of the complex molecule of nucleic acid, it is necessary to understand how the components of the mononucleotide unit are synthesized.

Phosphate is readily available from the phosphate pool of the body.

Ribose and deoxyribose are formed during the metabolism of glucose in the HMP pathway.

It is only necessary to consider here how the pyrimidine and purine rings are synthesized. In fact, both these are synthesized along with the entire nucleotide in a stepwise manner.

Synthesis of pyrimidine nucleotides:

1. *Formation of carbamyl aspartate:* Carbamyl phosphate is first formed from CO_2 and NH_3 by the action of carbamyl phosphate synthetase. The reaction is similar to the first step in urea synthesis. But while the liver enzyme uses free ammonia and requires N-acetylglutamic acid as a co-factor the enzyme which leads to pyrimidine biosynthesis is present in all tissues and utilizes amide nitrogen of glutamine as source of the $-NH_2$.

$$\text{Glutamine} + CO_2 + 2\,\text{ATP} \longrightarrow NH_2CO\sim P + \text{glutamate} + 2\,\text{ADP} + P$$

The carbamyl phosphate now combines with aspartate to form carbamyl aspartate. This is a "committed step" in pyrimidine biosynthesis. It is subject to feed back inhibition by CTP or UTP, the end products.

2. *Formation of dihydro-orotic acid:* The carbamyl aspartate loses a molecule of water to form dihydro orotic acid. (See fig. 16-1).

3. *Formation of uridylic acid:* On removal of 2 hydrogens, orotic acid is formed. The orotic acid is then decarboxylated and combined with phosphorylated ribose to form uridine-5'-phosphate (uridylic acid or UMP). UMP can be phosphorylated further by ATP to form UDP and UTP.

Thus the N1 and N2 are derived from carbamyl phosphate and N3 and C4, C5 and C6 from aspartic acid. Uracil is the pyrimidine base first synthesized. It can be aminated in position (4) to form the cytosine derivatives. The amino group of glutamine contributes the NH_2 to the 4th carbon to form cytidilic acid.

The uracil moiety can be methylated in position 5 in the presence of serine (donor of $-CH_2O$ group), tetrahydrofolic acid (carrier of the $-CH_2O$ group), ATP and Mg^{++} to form thymine. B_{12} is required in the final reduction of the formyl group to a methyl group.

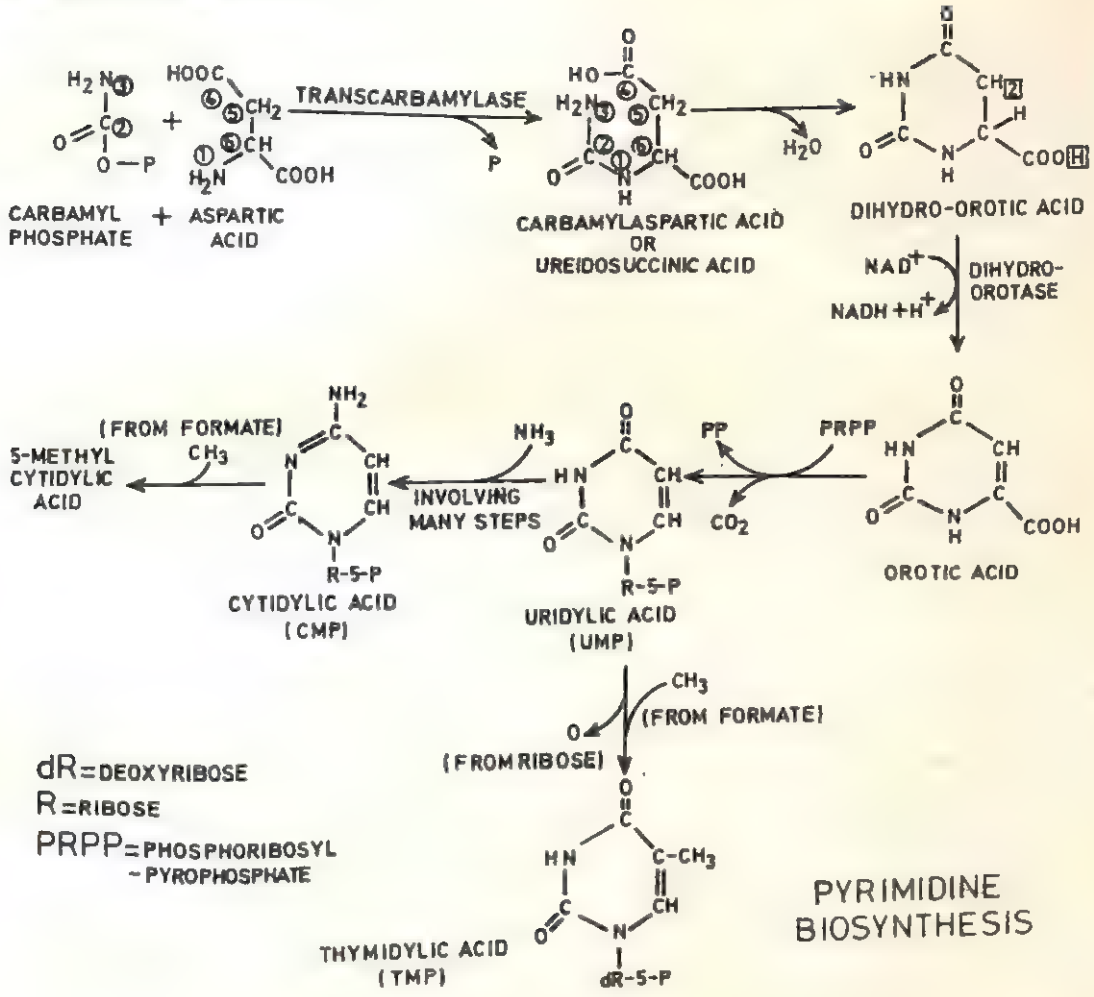


Fig. 16-1. Biosynthesis of pyrimidines.

There is a simultaneous reduction of the ribose in the second carbon (removal of 'O' from the CHOH to form CH_2) to produce deoxyribose which is the constituent of thymidilic acid (DNA nucleotide). This requires vitamin B_{12} and a sulfhydryl protein called "thioredoxin" as coenzymes.

Orotic aciduria: This is a hereditary disorder caused by a deficiency of the enzymes that convert orotic acid to UMP. The child shows stunted growth and megaloblastic anemia. Administration of uridine or cytidine will restore normalcy.

Pyrimidine catabolism: The products of breakdown of the pyrimidine ring are urea, ammonia and carbondioxide. This can occur after release of the pyrimidine ring from the nucleotide combination or even while it is still in the nucleotide. By a reversal of the steps of synthesis, cytosine forms uracil which gives rise to β-ureido-propionate, and 5-methyl cytosine and thymine give rise to β-ureidoisobutyrate. They are ultimately converted to NH_3 and CO_2. (See fig. 16-2).

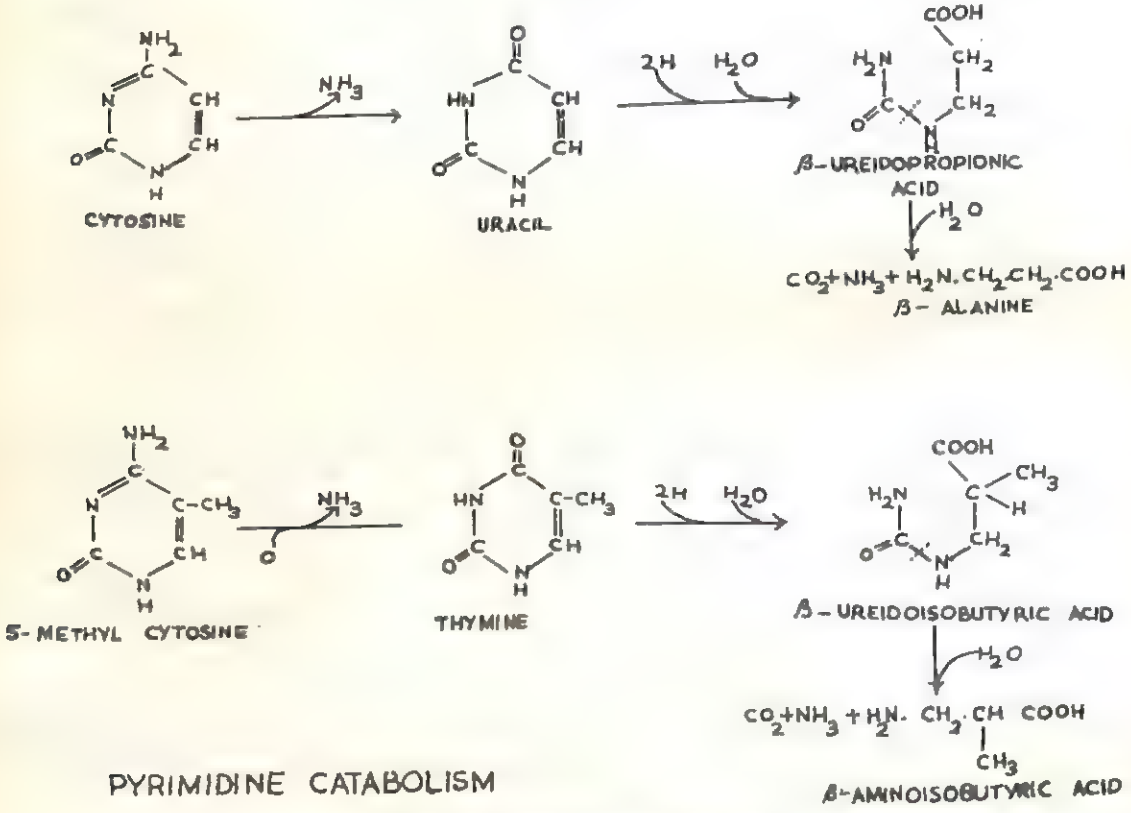


Fig. 16-2. Catabolism of pyrimidines.

The CO_2 and NH_3 join their respective pools and may form urea or any other substance. The β-alanine may be used for pantothenic acid synthesis or be oxidized. The β-amino isobutyrate is also similarly oxidized.

Biosynthesis of the purine ring: (Fig. 16-3). The purine ring, like the the pyrimidine ring, is synthesized along with the nucleotide. Inosinic acid (containing hypoxanthine as the purine base) is formed first.

1. *Formation of 5-phosphoribosy-l-pyrophosphate (PRPP):* Ribose is converted by successive phosphorylations of C_1 and C_5 to form 5-phosphoribosyl-l-PP. ATP is required as phosphate donor.

2. The pyrophosphate in C_1 is replaced by an NH_2 group from glutamine to form 5-phosphoribosyl-l-amine. The formation of 5-phosphoribosy-l-amine is a "committed step" in purine biosynthesis. It is subject to feedback inhibition by purine nucleotides. The reaction is also inhibited by azaserine.

3. Glycine combines with the above compound to form glycinamide ribotide. The positions which each of the carbons and nitrogens are to occupy in the final purine ring are appropriately numbered in fig. 16-3.

4. A formyl group is added to the N at position (7) to form formyl glycinamide ribotide. This step requires tetrahydrofolic acid.

5. Glutamine will now add the NH_2 of position (3) and closure of ring occurs betwee n C_8 and N_9 to form formyl-glycinamidine-ribotide.

6. Carbondioxide (from CO_2 pool of the body) and NH_2 from aspartic acid are added as carbamate to C_5 to form the C_6 and N_1 and the compound now formed is 5-amino-4-imidazole-carboxamide-ribotide. This step requires biotin.

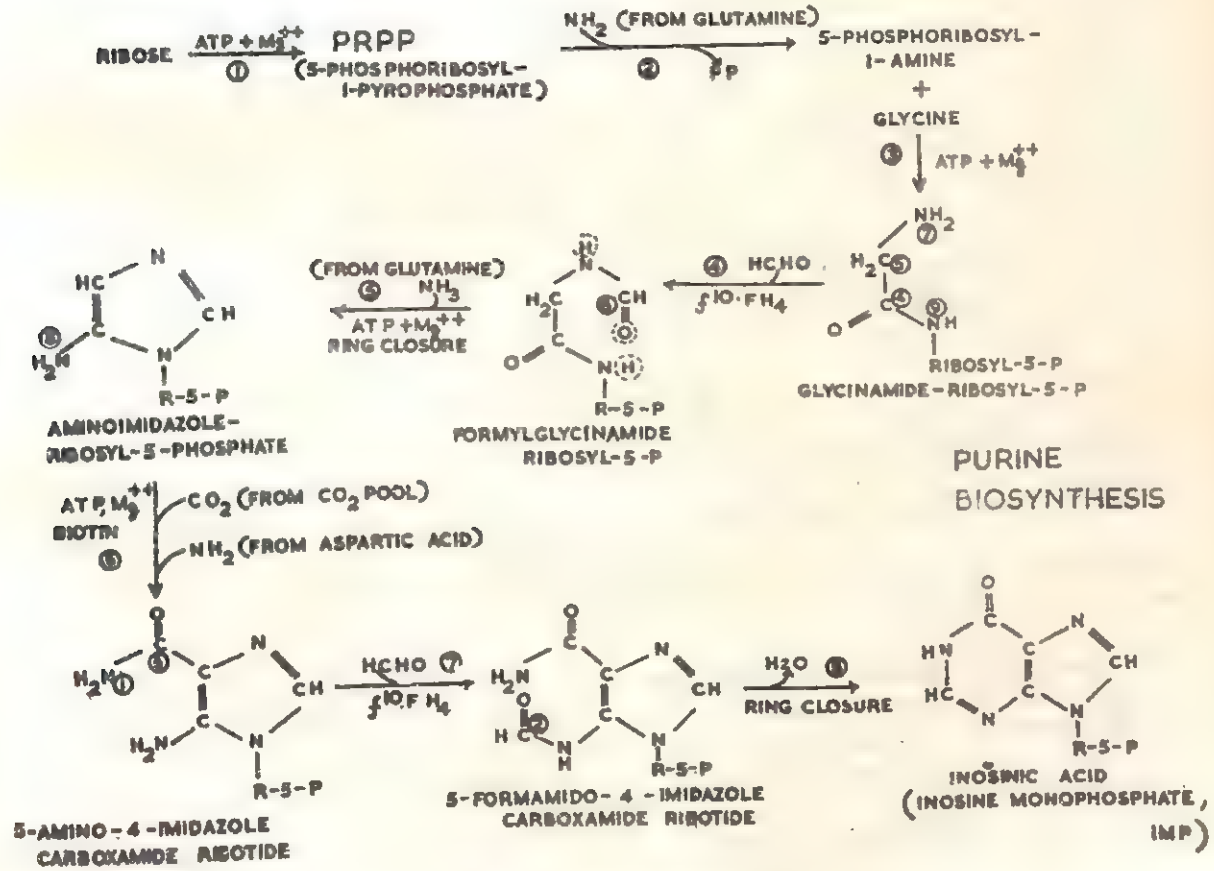


Fig. 16-3. Bisoynthesis of purines.

7. A formyl group is now added to the amino group of N_3. This step again requires tetrahydrofolic acid. The intermediate now formed is 5-formamido-4-imidazole-carboxamide-ribotide. The formyl C will be the future C_2 of the purine ring.

8. Ring closure now occurs between N_1 and C_2 to form inosine monophosphate (IMP) or inosinic acid.

9. Inosine is converted to adenine by taking an amino group at C_6 from aspartic acid.

10. Inosine can be converted to guanine by oxidation of C_2 to C=O and later amination from glutamine to form $C.NH_2$. Reactions 9 and 10 occur while still in the nucleotide (as inosinic acid). Hence the product formed in step 9 is adenylic acid (AMP) and in step 10 it is guanylic acid (GMP).

The sources of each of the carbons and nitrogens of the purine ring in the above complicated synthetic process are summarized in fig. 16-4.

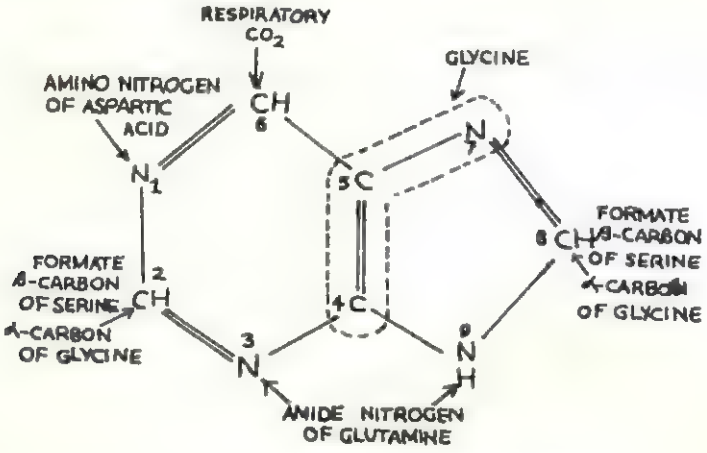


Fig. 16-4. Sources of N and C of the purine ring.

The insertion of C_2 and C_8 require tetrahydrofolic acid as carrier for 1-carbon moiety.

Both carbons of glycine and its nitrogen enter into positions C_4, $C_5$and N_7 of the purine ring.

11. The purine ribonucleotide is converted to deoxyribonucleotide by reduction of the second carbon of ribose. The reaction requires a protein cofactor called 'reduced thioredoxin' which is converted to the oxidized form in the reaction. It is converted to the reduced form again by the enzyme 'thioredoxin reductase' using $NADPH+H^+$ as coenzyme.

The erythrocyte, the polymorphonuclear leukocyte and the mammalian brain do not have the ability to synthesize the purine base. They depend on exogenous supply. The liver supplies the purine bases to these tissues.

Inhibitors of purine synthesis: Several compounds act as antimetabolites and inhibit purine synthesis. Azaserine, deoxynorleucine (DON), amethopterine (folic acid antagonist), purinethol and hadacidin are some and are used in the arrest of tumour growth, leukemias and other conditions.

Purine catabolism: The end product of purine metabolism in primates including man and the Dalmatian dog is uric acid. In the lower animals, birds, and reptiles, this is further broken down by the enzyme uricase to form allantoin and other products. The oxidation of the purine ring can occur while it is still in nucleotide combination or nucleoside combination. Liver, spleen, kidney and intestinal mucosa contain enzymes capable of acting on the purine ring in the free or combined state. The detailed steps are shown in fig. 16-5.

Adenase is absent in human. Instead, adenosine deaminase will convert adenine to hypoxanthine while in nucleoside combination. similarly adenylic acid deaminase will act while in nucleotide combination.

Adenylic acid —(Deaminase)→ Inosinic acid.

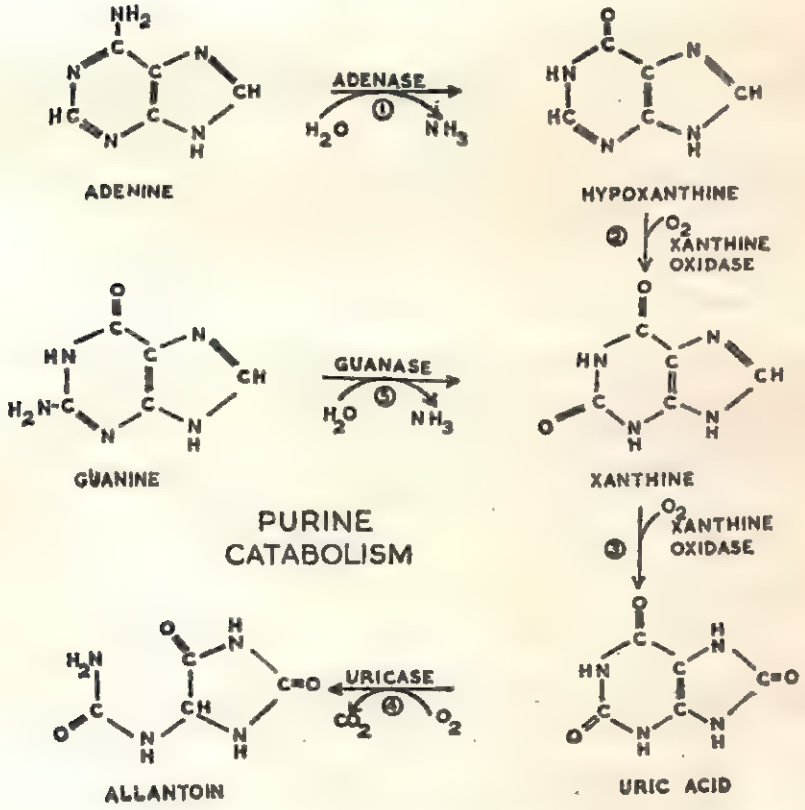


Fig. 16-5. Catabolism of purines.

Xanthine oxidase is present in several tissues and also in milk. It contains riboflavin as prosthetic group and requires molybdenum and iron as activators. An inborn deficiency or low levels of the enzyme cause excretion of large amounts of xanthine in urine (xanthinuria). Xanthine stones may be formed in the urinary tract.

Uric acid metabolism:

In a normal adult, the plasma contains 2–6 mg/100 ml. The blood levels do not depend on food intake of purines. There seems to be an inverse relation between the amount of purines synthesized and the food intake so that a homeostasis is maintained. The miscible pool of uric acid is about 1200 mg. in man and about 600 mg. in woman. Uric acid is mainly excreted in urine by glomerular filtration. A part of it is reasorbed by the renal tubule. Small amounts are also actively secreted by the renal tubule. Uric acid excretion in man is about 400–600 mg. in 24 hours. Foods such as milk, cheese and eggs are low in purine content. Liver, thymus and organo-meats are rich in purines.

About a fifth of the uric acid is excreted through bile into the intestines and is broken up to CO_2 and NH_3.

Gout: In this condition, the blood levels of uric acid are increased and also abnormal deposition of uric acid crystals occur in joints, tendons and bursae leading to painful condition of these structures. The deposits are called tophi. The uric acid pool is increased to 5,000 mg. or more.

Primary gout: It is a metabolic disorder in which the kinetics of the enzyme 'PRPP' synthetase' are altered leading to overproduction of PRPP and more of purine synthesis.

It may also occur secondary to an increase in purine catabolism in conditions such as leukemias or secondary to renal failure. This is known as *secondary gout.*

Certain substances like salicylates and cinchophen and also the adrenal cortical hormones (ll-oxysteroids) cause an increase in the urinary excretion of uric acid by decreasing its reabsorption. These are called uricosuric drugs and are useful in the treatment of gout. Allopurinol is another substance, which, by its structural resemblance to hypoxanthine, competitively inhibits xanthine oxidase, and decreases the production of uric acid.

In blood plasma, uric acid mostly exists as sodium urate. It has a maximum solubility of about 6.8 mg/100 ml. In acidic urine (pH about 5.4) uric acid and urate occur in equal amounts.

Uric acid is insoluble in acid and neutral urine and may be precipitated and form stones (calculi) in the urinary tract.

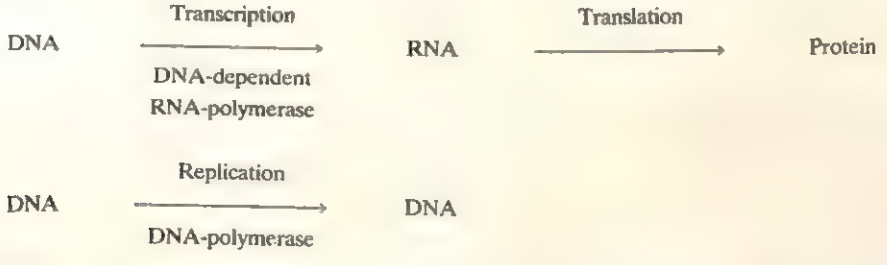
In von-Gierke's disease also, there is overproduction of uric acid. Due to deficiency of glucose-6-phosphatase, the HMP shunt pathway is overactive and produces excessive amounts of ribose-5-phosphate. This leads to overproduction of PRPP and, hence, uric acid.

Lesch-Nyhan Syndrome: There is a hereditary deficiency of a salvage enzyme -hypoxanthine phosphoribosyl transferase - which salvages purine bases by converting them to their corresponding nucleotides. The enzyme is particularly useful in the central nervous system where de novo synthesis to purine bases is limited and exogenous supply is needed. A deficiency of the enzyme leads of oxidation of the bases to uric acid. It gives rise to cerebral palsy, involuntary movements and a tendency to self mutilation in the affected children.

Hypouricemia: This may be due to diminished reabsorption from the renal tubules or deficiency of the enzyme 'xanthine oxidase'. There is an increased excretion of hypoxanthine and xanthine. Deficiency of purine nucleotide phosphorylases will result in excretion of purine nucleosides in urine and diminished blood uric acid levels.

Adenosine deaminase deficiency is associated with an immuno-defieiency disease where the T cells and the B cells are both decreased in number and function. Purine phosphorylase deficiency is also associated with a form of immunodeficiency disease. These deficiencies are inherited as autosomal recessive. There is intracellular accumulation of triphosphates like deoxy-ATP and deoxy-GTP and an inhibition of DNA synthesis in the affected cells (eg. T cells).

Biosynthesis of nucleic acids: Nucleic acids are formed by polymerization of several nucleotides. While DNA can guide its own synthesis, RNA cannot. It is guided by DNA. The synthesis of additional DNA is known as 'replication' and is brought about by the enzyme 'DNA-polymerase' while the synthesis of RNA is known as transcription and is brought about by a DNA dependent 'RNA-polymerase'.



For each gene in the DNA molecule, there is a *'sense'* strand and its complementary *'antisense'* strand. The diploid genome of human cells has a molecular weight 3×10^{12} and is made up of 23 pairs of chromosomes.

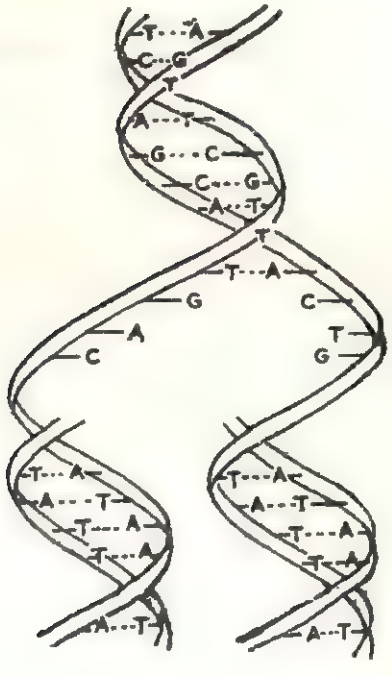
DNA Synthesis and Replication:

This was first studied in E.Coli by Arthur Kornberg. The enzyme concerned with the synthesis is now called DNA polymerase I. The dexoyribonucleotidetriphosphates of adenine, guanine, cytosine and thymine (dATP, dGTP, dCTP and dTTP) are required for the synthesis.

Before a DNA strand can serve as template for its own synthesis, the two strands in the double helix have to unwind and separate in the region which has to be replicated.

Semiconservative Replication of DNA: Each single strand of the double helix of the DNA will serve as a template to form a new DNA strand complementary to itself. When the process is complete, two double stranded molecules are formed, each of them containing one original strand and one newly synthesized complementary strand (see Fig. 16-6).

The synthesis of DNA is initiated by the formation of a short RNA molecule of 10 nucleotides. The first deoxyribonucleotidetriphosphate is attached to 3^1-hydroxyl end of RNA with the cleavage of the extra two phosphates as pyrophosphate. To this is added the second deoxyribonucleotidetriphosphate, again a pyrophosphate is cleaved from the triphosphate, and the process is repeated stepwise. To guide each deoxyribonucleotide into position, one of the pre-existing DNA strands (sense or antisense) will act as template. Against ATGC of the pre-existing strand, TACG will be introduced in the new DNA strand. *i.e.*, the two strands are complementary to each other. Pieces of DNA containing about 100 nucleotides are synthesized at a time. The DNA containing about 100 nucleotides and attached to a small RNA molecule of 10 nucleotides is called an *"Okazaki"* piece, after the person who discovered it. A number of these pieces are then united after removing the RNA pieces by the enzymes DNA-ligases. The same set of enzymes can act on both the original DNA strands (sense and antisense) by turns to produce two daughter strands of DNA complementary to each other and complementary to their respective parent strands. (See Fig. 16-6). The process is known as *replication*. Replication in mammalian cells must be similar in nature.



The two strands of DNA separate out and each serves as template for replication of a new DNA strand.

Fig. 16-6. Replication of DNA.

In mammalian cells, a number of enzymes are used to facilitate replication of the DNA strands. The DNA polymerase of mammalian cells is 10 times less active than its counterpart in bacteria. Each portion of the DNA strand between neighbouring nucleosomes is separately synthesized as Okazaki pieces and then annealed together to form a single long strand by enzymes called DNA ligases. The process of unwinding of the two strands of the parent DNA helix is also hastened by enzymically nicking the strand in several places (the same when one tries to unwind a roll of twine which is very much entangled). The nicks are resealed again by enzymes, *DNA topoisomerases*. A detailed description of these enzyme processes is beyond the scope of this book.

Mechanisms also exist in the nucleus to repair, replace or otherwise change bases which are found to be abnormal. Thus, spontaneous deamination of cytosine or adenine will result in their conversion to uracil or hypoxanthine in the DNA molecule. Such abnormal bases are recognized and removed and the correct bases are reintroduced by appropriate enzymes. Lack of function of some of these repair mechanisms gives rise to certain inherited diseases like Xeroderma Pigmentosum, Ataxia-Telangiectasia and Fanconi's anemia.

Z-DNA: A form of DNA called the Z-DNA has been discovered recently (1980). It is a left handed double helical structure and the phosphodiester backbone zig-zags along the molecule (hence the name, Z-DNA). It is more stretched and hence thinner than the regular DNA (the B-form) and has alternate pyrimidine and purine bases in each chain (say cytosine and guanine). Cytosine is often methylated. The Z-DNA forms short stretches between long stretches of regular DNA. It probably exerts a regulatory effect on gene activity and protein binding. Loss of methyl groups from cytosine can revert the Z-form to the B-form and cause some torsion of the DNA molecule distal to the changed molecule. This seems to have an influence on gene activity.

Genetic Engineering: Recombinant DNA experiments:

Genetic engineering or *'cloning'* is the introduction of genetic material from one organism into another, where it may be expressed.

It has become possible now to synthesize in the laboratory specific segments of DNA molecules which code for specific proteins, say one of the protein hormones. These DNA segments can be introduced into bacteria and made to combine with their circular DNA. When such bacteria multiply, the new gene is replicated and transferred to the progeny. By culturing these bacteria, large amounts of the new protein (eg. insulin) can be harvested. Bacterial enzymes called "Restrictive Endonucleases" are found to be very useful in cleaving the phosphodiester backbone at specific symmetric sites in the DNA molecule. Small DNA molecules so produced can be recombined suitably with the same or other DNA molecules.

Viruses: They do not have any ribosomes or other subcellular organelles and are hence not capable of generating energy on their own. They depend on the host cell which they infect for this purpose. The virus particle attaches to the surface of a cell at specific receptor sites. The cell membrane is then penetrated either by the entire virus particle or only the nucleic

acid part of it. Viral proteins and nucleic acids are synthesized in the host cell on the direction of the viral nucleic acid. If it is a DNA virus, the usual processes of transcription and translation are followed. The viral DNA may remain separate in the cell or may get integrated with the cellular DNA by processes similar to genetic engineering.

There are some animal viruses capable of synthesizing DNA molecules complementary to an RNA template. The enzyme is called "RNA-dependent DNA-polymerase" and the process is called *"Reverse transcription."* A DNA strand complementary to the viral RNA is formed and incorporated into the DNA of the host cell chromosomes and thus maintains the transformed state of the host cells. Similar process is also seen to occur in some mailgnant cells like the leukemic cells.

Interferons: These are small glycoproteins (M.W. 26,000 to 38,000) produced by leukocytes and fibroblasts as a defence against viral infection. During replication of RNA viruses, a double stranded RNA is formed. This stimulates the production and release of interferon by the infected cell. In the presence of interferon, the translation process of the DNA virus is seriously interfered with by inactivating some of the initiation factors. Another mechanism of interferon action is by rapidly destroying the mRNA formed. Multiplication of the virus is thus arrested.

The replication of the DNA genome occurs only preceding the mitotic division of the cell. There is some regulatory mechanism in the cell which prevents such replication at other times.

Damage to DNA synthesis can occur due to several factors. Ultraviolet irradiation can cause hydration of the cytosine residues. Formation of dimers can occur between thymine and thymine of opposite strands through a cyclobutane bridge. The DNA chain can also be broken by X-ray irradiation, or radioactive isotopes.

RNA Synthesis (Transcription):

The sequence of ribonucleotides in an RNA molecule is complementary to the sequence of deoxyribonucleotides in one of the strands of the DNA template molecule. Only the *sense* strand can serve as a template for RNA synthesis.

The process of synthesis of RNA molecules from DNA templates is known as *"transcription."* The RNA molecules synthesized by transcription are 10–100 times larger than the mRNA molecules (which only contain 5,000 to 50,000 nucleotides). These large molecules are called "heterogenous nuclear RNA" (h_nRNA). The mRNA molecule has at its 3' end (tail end) a polyadenylic acid base sequence-poly-A. At its 5' end (cap) it has 7-methylguanosine-5'-triphosphate. The cap is probably added in the nucleus itself. The polv-A can be added either in the nucleus or in the cytoplasm.

The enzyme responsible for the synthesis is DNA-dependent-RNA-polymerase. The enzyme binds itself to the 3' end of the *sense* strand of DNA, the promotor site.

A specific protein factor - the sigma (δ) factor - assists the RNA polymerase enzyme to attach itself tightly to the promoter site. The polymerase can act on 17 base pairs at a time and has the capacity to unwind the double helical DNA to that extent. When done with, it can also rewind the double helix back again in steps of 17 base pairs. The sigma factor gets released from DNA soon after the transcription process commences.

The starting point of transcription (towards 3' end of DNA) corresponds to the 5' terminal of the RNA transcribed. The synthesis of the RNA molecule is terminated when a particular sequence in the DNA template is reached. This sequence is recognized by a specific protein called the (P) factor.

In the process of transcription, the *introns* also participate. But the hnRNA formed from these portions is cleaved and the functionl portions are spliced together in the nucleus and the functional mRNA enters the cytoplasm.

Failure to cleave at the extron-intron junction due to the presence of abnormal nucleotide at the junction results in the failure to release the appropriate mRNA for the synthesis of the beta chain of hemoglobin and causes a molecular disease - *"beta-thalassemia"*.

Ribonucleotide triphosphates (ATP, GTP, CTP and UTP) supply the nucleotides which are added in an order complementary to the base sequence in the template - A for T, G for C, U for A and C for G. Synthesis always starts with the introduction of a purine nucleotide - adenylic or guanylic acid. The RNA synthesis proceeds from 5' - end to the 3' end. A specific base sequence of the DNA strand will signal the termination of RNA synthesis. The enzyme will then separate from the promotor site.

The tRNA molecules, as formed in the nucleus by transcription, are larger and are subsequently cleaved to the actual size by specific ribonucleases. Methylation of some of the bases and addition of the -C-C-A terminal nucleotide at the 3' end also occur later.

Ribosomal RNA is also formed as aggregates of more than one ribosome and later cleaved to individual risosomes. The genes for the ribosomal RNA are located in the nucleoli of mammalian cells.

RNA polymerase Types I,II and III exist for transcribing ribosomal RNA, mRNA and tRNA respectively.

A toxin present in the mushroom *Amanita Phalloides* causes inhibition of all three polymerases to varying degrees and causes acute gastrointestinal symptoms like pain, nausea, vomiting and diarrhoea and in severe cases damages the liver and kidneys. The toxin is an octapeptide.

References:

Chargaff, E. and Davidson, J.N. (eds): The Nucleic Acids, Vols. 1,2 and 3., Acad. Pr., N.Y., 1955 and 60.
Davidson J.N.: The Biochemistry of the Nucleic Acids, 5th ed. Wiley, N.Y., 1965.
Harris, H.: Human Biochemical Genetics: Cambridge University Pr., N.Y., 1959.
Ingram, V.M.: The Biosynthesis of Macromolecules, Benzamin, N.Y., 1965.
Kornberg, A.: Enzyme Synthesis of DNA., Wiley, N.Y., 1962.
Steiner, R.F.: Chemical Foundations of Molecular Biology; Van Nostrand, Princeton, N.J., 1965.
Taylor, J.H. (ed).: Molecular Genetics, Acad. Pr. N.Y., 1967.
Wagner, R.P. and Mitchell, H.K.: Genetics and Metabolism, 2nd, ed., Wiley, N.Y., 1964.

17

BIOSYNTHESIS AND METABOLISM OF PROTEIN

PROTEIN is the structural component of protoplasm and is thus an important constituent of all cells and tissues. Every tissue has its own characteristic proteins. All enzymes and some of the hormones are also proteins. The proteins of plasma play an important role in the normal distribution of body fluids. Antibodies and the oxygen carrying substance hemoglobin are also proteins.

The amino acids, on absorption form the intestine, are taken up from the portal blood partly by the liver and the rest enter the systemic circulation to be taken up by all other tissues. They are utilized for the synthesis of the proteins of the tissues, enzymes and hormones and also provide energy by their break down. The nitrogen moiety is utilized for synthesis of other nitrogenous substances like creatinine and choline and the rest is converted to urea and excreted, while carbon skeleton is completely oxidizable to carbon dioxide and water. The sulfur contained in some of the amino acids and the phosphorous of phosphoproteins are converted to sulphate and phosphate and excreted in urine.

Dynamic equilibrium:

All tissues including blood have a constant composition and size in the adult. This is not on account of the stationary or static nature of the adult tissues. On the other hand, there is a constant breakdown of the tissues and a replenishment by resynthesis. In a normal adult who is neither gaining nor losing weitht, the breakdown and synthesis balance each other, thus keeping the composition and size of the tissue constant. This is known as 'Dynamic Equlibrium.'

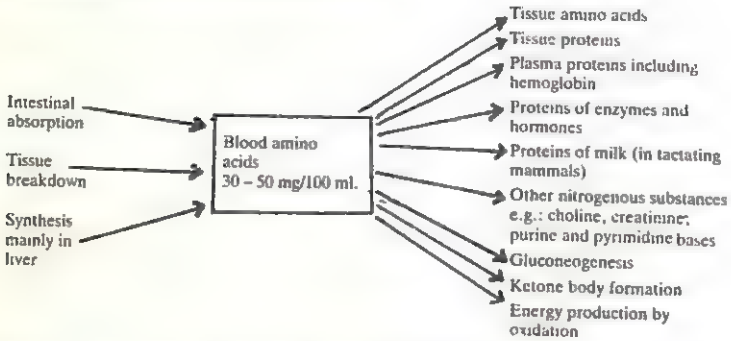
Thus proteins of every tissue including blood are continuously being broken down to amino acids. There is also a continuous synthesis of amino acids (excepting the essential amino acids) and the periodic absorption of amino acids from the intestines. Amino acids from all these sources get mixed up to constitute what is known as the 'amino acid pool' of the body. All tissues including exocrine and endocrine glands draw freely from this amino acid pool to synthesize the tissue proteins, enzymes and protein hormones.

In an adult 70 kg man, about 400 grams of protein is synthesized daily and that much is also degraded.

The concept of dynamic equilibrium as originally proposed by Whipple relates to the exchangeability of the amino acids between plasma proteins and tissue proteins.

These concepts of dynamic equilibrium and metabolic pool are equally applicable to carbohydrates, lipids, minerals and all other constituents of the tissues.

Sources and utilization of blood amino acids



Nitrogen balance: In an adult healthy animal maintaining constant weight, the amount of intake of nitrogen in food (mainly as protein) will be balanced by excretion of an equal amount of nitrogen in urine (in the form of urea mainly; uric acid, creatinine, creatine and amino acids contribute to a minor extent) and feces (mainly unabsorbed nitrogen). The animal is said to be in nitrogen balance. In the growing period and also during convalescence from illness when the animal is putting on weight, the nitrogen intake will be more than the output, since some of the nitrogen is retained as tissue protein. This is known as positive nitrogen balance. The reverse occurs in old age and during illness resulting in a negative nitrogen balance.

There is no special storage form for protein like glycogen for carbohydrate or adipose tissue for fat. Protein storage is always accompanied by tissue growth. However, during starvation, when protein is not available from dietary sources, it is the liver which loses the largest proportion of its protein compared to other tissues. Additions of protein in food similarly cause an appreciable increase in liver weight first. Thus the liver protein appears to be more labile than the protein of other tissues. Kidney and blood proteins come next in degree of lability.

Amino acids of blood: All the amino acids occur in blood in varying concentrations and make a total of 30–50 mg/100 ml in the postabsorptive state. In terms of amino acid nitrogen it is 4 to 5 mg/100 ml. Following a protein containing meal, the amino acid levels rise to 45–100 mg/100 ml (amino acid nitrogen 6–10 mg/100 ml).

Tissue amino acids: The amino acids are actively transported into tissues. Pyridoxal phosphate is one of the requirements for the normal uptake. Growth hormone, insulin and testosterone favour the uptake of amino acids by tissues. Estradiol selectively stimulates their uptake by uterus. Similarly epinephrine and glucocorticoids stimulate the uptake of amino acids by the liver. The quantity of amino acids present at any given moment is the sum total of the absorbed amino acids and those derived by the breakdown of tissue. During starvation, they may actually be increased due to an increased tissue breakdown though there is no absorption from outside.

Different tissues exhibit preference in the utilization of particular amino acids. In the postabsorptive state, plasma amino acids consist mostly of alanine and glutamine. The intestines take up with avidity glutamine, glutamic acid, aspargine and aspartic acid and release alanine into the blood stream. Alanine is also released by the skeletal muscle and kidney. Liver takes up the alanine and utilizes it for gluconeogenesis (alanine-glucose cycle) Branched chain amino acids — valine, leucine, and isoleucine — are oxidized in large amounts by the skeletal and cardiac muscles. Brain also utilizes branched chain amino acids — particularly valine. Kidneys take up glutamine, proline and glycine and release alanine and serine.

PROTEIN SYNTHESIS

The knowledge of the detailed mechanisms of protein synthesis has advanced by leaps and bounds during recent years and is continuing to progress still. Using automated techniques, the A and B chains of insulin (21 and 30 amino acid residues each) and the pancreatic ribonuclease (124 amino acid residues) have been synthesized. Only a simple outline of the mechanisms of protein synthesis will be attempted in this chapter.

The synthesis of the polypeptide chain of a protein seems to occur by a stepwise incorporation of the individual amino acids and not by simultaneous inclusion. Yet the process is so rapid that the synthesis of the peptide chain of hemoglobin can be completed in 2½ minutes.

Site of synthesis: The bulk of protein synthesis takes place in the ribosomes of the endoplasmic reticulum. Other structures like nuclei and mitochondria can synthesize small amounts of special proteins required for themselves. Ribonucleic acid plays a very active role in the entire process and guides the amino acids to their respective places in the polypeptide chain.

Messenger RNA: It is ultimately the genes, which are DNA molecules in the chromosomes, that determine the type of protein to be synthesized by the cell. In fact, for every protein synthesized, there is said to be a specific gene guiding the process. Since the synthesis is occurring in the ribosomes which are located in the cytoplasm, the message is carried by a molecule of RNA from the gene (DNA) to the ribosome. This is called the messenger RNA. While the DNA, tRNA and ribosomal RNA are constantly present, mRNA exists only transiently and does not accumulate.

On a signal from the cytoplasm one of the two strands of the DNA of the gene is said to be activated (or a repressing influence is removed). It now functions as a template for the synthesis of a complementary RNA strand in juxtaposition to itself such that bases of RNA correspond to the bases of DNA as shown on page 336. This RNA molecule is called the messenger RNA or mRNA and forms with DNA strand a 'hybrid helix.'

Gene DNA	..	*Messenger RNA*
Adenine (A)	..	Uracil (U)
Guanine (G)	..	Cytosine (C)
Thymine (T)	..	Adenine (A)
Cytosine (C)	..	Guanine (G)

A strand of DNA is capable of directing the synthesis of several different proteins. The portion of DNA strand which is concerned with the synthesis of any single polypeptide chain is called a 'cistron'.

The replication of a messenger RNA from the DNA of the gene is described as '*transcription*' and is mediated by the enzyme '*RNA polymerase II* .

The polyribosome: The mRNA so formed in the nucleus now migrates into the cytoplasm and attaches itself to a number of ribosomes. This complex structure is called a polyribosome, polysome or ergosome. The ribosomes can move along the length of the mRNA strand and seem to activate the mRNA by their presence. The ribosomes have a molecular weight of about 2.5 millions. In the E-Coli, they have a sedimentation constant of 70S and can be further separated by ultracentrifugation into two components of 50S and 30S with molecular weights of 1,100,000 and 500,000. (In the mammalian cells, the corresponding ribosomal components are 60S and 40S). An average number of ribosomes in a polysome is five. It is the 30S component that gives attachment to the mRNA.

We have now a mould or template ready to take up amino acids in a specific sequence and form a polypeptide. Which amino acid is to be taken at which point of the template seems to be determined by some sort of coding on account of the sequence of the purine and pyrimidine bases in the messenger RNA. This role of mRNA in directing the amino acid sequence in protein synthesis is referred to as 'Translation'.

The Genetic code: In case of DNA, the four bases, adenine, guanine, thymine and cytosine (A, G, T, C), can form 64 different three base combinations (triplets) — AAA, AAG, AAT, AAC, AGA, ATA, ACA, and so on. Each triplet represents the code word for an amino acid. There may be more than one triplet for each amino acid and several triplets are probably without function. In replicating a complementary RNA strand, the messenger RNA carries the code given in the table. (See Table 17-1).

The deciphering of the genetic code, we owe mainly to the work of Nirenberg and Matthaei (1961). This was made possible by the nucleotide polymers and nucleotide triplets synthesized by Khorana.

Nirenberg and Matthaei (1961) experimented with cell-free E. Coli extracts, freed from DNA by treatment with DNA-se. When a synthetic polynucleotide — poly (U) (polynucleotide containing only uridylic acid) — was added to the medium, a polypeptide containing phenylalanine units (polyphenylalanine) was obtained. Similarly poly (A) formed polylysine and poly (C) formed polyproline. Hence UUU codes for phenylalanine; AAA for lysine; and CCC for proline.

The experiments were enlarged using mixed polynucleotides and the entire genetic code was unravelled.

Khorana and his associates synthesized polynucleotides with two alternate bases e.g. CUCUCU.. This resulted in the formation of a polypeptide containing leu—ser—leu—ser—leu—ser.... This confirms not only that CUC codes for leucine and UCU for serine; but it also confirms triplet coding. If the code were to be two or four bases — CU CU CU CU CU .. or CUCU CUCU CUCU .., it would have resulted in a polypeptide containing a single amino acid.

The genetic code is the same for all the organisms, *i.e.*, it is *Universal*. The first two bases in the triplet are the more important. Some amino acids are represented by more than one codon — degeneracy of the code. But each codon represents only one amino acid. The genetic code is thus unambiguous.

The genetic code can be overlapping or non-overlapping. Thus in a 6 base sequence of AGCTAG, if the code is overlapping, the base C can be one of any of the following triplets — AGC, GCT, CTA — and thus code for 3 different amino acids. In the non-overlapping code, it forms only one triplet AGC and codes for only one amino acid. In mutation, alteration of the gene in one base will only result in replacement of a single amino acid. The coding is therefore non-overlapping.

Each triplet called the 'Codon' provides specific binding for a specific amino acid carrier. Thus, UUU and UUC can take up the carrier molecules of phenylalanine and UUA and UUG can take up that of leucine. What remains now is for the amino acids to be taken and deposited at their appropriate positions on the template.

Aspargine and glutamine have codons different from those of aspartic acid and glutamic acid. There are codons for cystein, proline and lysine, but none for cystine, hydroxyproline

TABLE 17-1.

The Genetic Code

First base	Second base. U	C	A	G	Third base
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	*Term.*	*Term.*	A
	Leu	Ser	*Term.*	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met (CI)	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val (CI)	Ala	Glu	Gly	G

Gln — glutamine, Asn — aspargine; Trp — tryptophan; *Term.* — nonsensory or terminator codon, CI — Chain initiating codon.

and hydroxyline. Conversion to these derivatives occurs after formation of the polypeptide chain. There are minor changes in some of the codons involved in the mitochondrial protein synthesis. In them,

AGA and AGG .. Term

AUA .. Met_f and UGA .. Trp.

Activation of Amino acids: The soluble fraction of cytoplasm contains several enzymes that are precipitated at pH 5.0 (the pH 5 fraction) which are called aminoacyl-sRNA synthetases. There is an enzyme for each of the amino acids.

$$\text{Amino acid} + \text{ATP} + \text{Enzyme} \xrightarrow{Mg^{++}} \text{Amino acid} - \text{AMP} - \text{Enzyme complex} + \text{PP}$$

Formation of sRNA – Amino Acid complex: In the cytoplasm, a form of RNA molecules of relatively low molecular weight (20,000 to 30,000) containing only 60 to 90 nucleotides are present. These are soluble and they take up the amino acid from the activated aminoacyl-AMP-enzyme complex and transfer them to their appropriate place on the polyribosomal template. These small RNA molecules are called the soluble RNA (sRNA) or transfer RNA (tRNA) molecules. Adenine, guanine, cytosine and uracil are the main bases as in the case of RNA in general. But small amounts of certain unusual bases like pseudouracil, methylated adenine and guanine, thymine and 5-methylcytosine also occur. Many of the individual tRNA (or sRNA) molecules were studied and their exact nucleotide sequence determined. Thus, alanyl-tRNA has 77 nucleotide residues and a molecular weight of 26,600. The nucleotides are arranged in a cloverleaf pattern (See fig. 17-1).

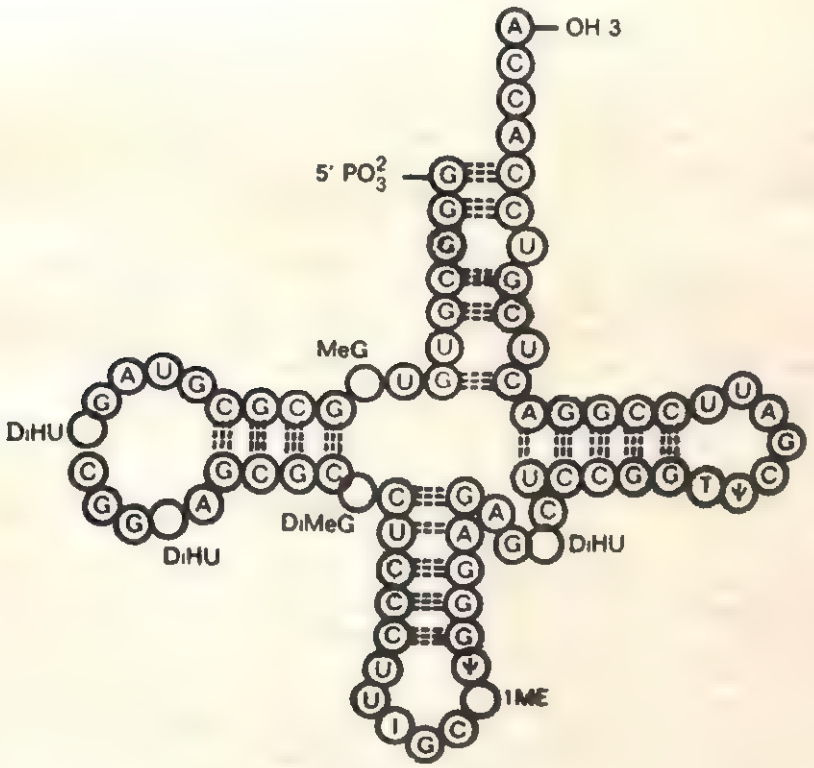


Fig. 17-1. Alanyl — tRNA

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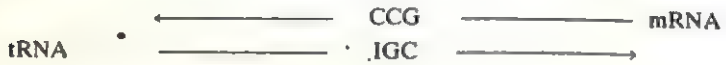
The dihydrouridine arm (on the left in Fig. 17-1) is probably the recognition site for the specific aminoacyl synthetase enzyme.

There is a variable arm or loop which varies in length from one tRNA to another. It can be seen as a small bump to the right in Fig. 17-1.

There is a larger bump (also to the right in fig. 17-1), the thymidine-pseudouridine-cytidine loop, which is the probable site of recognition for the ribosome.

The anticodon triplet IGC (inosine-guanosine-cytosine) occupies the convex portion of the leaf opposite to the open end of the structure. Inosine acts for guanine. Hence IGC anticodon locates and attaches itself to CCG, the codon for alanine.

The sequence in the anticodon loop is read from 3' to 5' end. But the sequence in mRNA and the genetic code is from 5' to 3'. Thus the anticodon of the tRNA and the codon of mRNA are antiparallel in the complementarity.



Due to degeneracy of the code — more than one codon coding for one amino acid, with the third base being of lesser consequence than the first two — it becomes necessary for the anticodon on the tRNA molecule to be less choosy as far as the third base is concerned. The complementarity of the bases between the codon on the mRNA and the anticodon on the tRNA is strictly followed with respect to the first two bases of the triplet, but discrimination is used with respect to the third base. This phenomenon is known as *"wobble"*

One end of the tRNA molecule invariably terminates in the base sequence CCA-OH (cytidylic acid, cytidylic acid, adenosine). The terminal base is only in nucleoside combination (*i.e.*, without phosphoric acid) unlike the rest which are all in nucleotide form. The adenosyl moiety can take up the specific amino acid, the –COOH of which combines with the –OH at position 3 of ribose.

$$\text{E-AMP-Amino acid+sRNA} \xrightarrow{\text{synthetase}} \text{E+AMP+sRNA–amino acid complex}$$

The amino acid molecules brought together on the template combine by peptide linkages to form the polypeptide chain and the sRNA molecules are released once again into the cytoplasm. The peptide linkages appear to start forming from the n-terminal end of the chain. Some of the codons act as 'chain initiating codons' and mark out the commencement of the polypeptide chain, while certain others act as 'chain terminator codons'. The initiator codon in case of E-Coli is AUG. It gives attachment to the sRNA carrying N-formyl-methionine. (The tRNA carrying methionine is designated $tRNA^{Met}_{m}$ and contains the usual thymidine-psuedouridine-cytidine sequence in the large bump to the right. The tRNA carrying N-formyl-methionine is designated $tRNA^{Met}_{F}$ and lacks this sequence in the bump. Though the anticodon is the same for methionine and N-formyl-methionine, (AUG), due to this difference in the structure of the bump, correct choice of methionine or its formyl derivative is made possible.

Simultaneously, a 50S ribosome gets into apposition to the 30S ribosome. The 50S ribosome carries two distinct binding sites — one called the 'peptidyl binding site' and the other called the 'aminoacyl binding site.' The N-formyl-methionine-sRNA is taken up at the peptidyl binding site. The $-NH_2$ of this amino acid is not free, but the –COOH is. The sRNA containing the n-terminal amino acid is taken up next at the aminoacyl binding site. A peptide bond is formed between the –COOH of formylmethionine and the $-NH_2$ of the n-terminal amino acid.

For the inititiation of the biosynthesis of a polypeptide chain, 'Initiation Factors IF_1, IF_2 and IF_3' are required. GTP is also required. They help in bringing together the 30S and 50S ribosomes and in inserting the tRNA carrying the n-formyl-methionine at the peptidyl binding site of the 50S ribosome. The tRNA for n-formylmethionine is different from that for methionine. The addition of each subsequent amino acid requires energy obtained by the breakdown of GTP to GDP and also factors known as 'Elongation Factors (EF).'

Now the ribosome moves along the mRNA strand to bring the next codon into the aminoacyl binding site. The n-terminal aminoacyl-sRNA moves to the peptidyl binding site and the formylmethionine-sRNA is freed from the ribosome. The sRNA separates out from the formylmethionine. A second aminoacyl sRNA now takes up the aminoacyl binding site on the 50S ribosome. A peptide bond is now formed between the –COOH of n-terminal amino acid and $-NH_2$ of the second amino acid.

The ribosome moves again one step on the mRNA, the n-terminal aminoacyl-sRNA gets out of the ribosome, the second aminoacyl-sRNA moves to the peptidyl binding site and the third aminoacyl-sRNA is placed in the aminoacyl binding site.

The process is repeated till the complete polypeptide chain is formed. The termination of the polypeptide chain is signified by the occurrence of a nonsensory or terminator codon (UAA, UAG, or UGA) on the mRNA. Since this does not code for any amino acid, the polypeptide chain is released from the last aminoacyl sRNA which carries the C-terminal amino acid. The ribosome pair is also released (See fig. 17-2).

The n-formylmethionine which inititated the polypeptide chain will get released at some stage during the above process, leaving the n-terminal amino acid as the first amino acid. When the ribosomes carrying the forming polypeptide chain move sufficiently down the mRNA strand, another set of 30S and 50S ribosomes will take up position at the initiator codon (n-formylmethionine) and start synthesis of a second polypeptide chain. At any given time, the mRNA strand will therefore carry several ribosome pairs to which are attached polypeptide chains of varying lengths — shortest near the initiator codon and longest near the terminator codon.

The ribosome pairs and the sRNA molecules on release from the mRNA can again function, in repeating the process.

Synthesis of polypeptide chain as described above is fairly rapid. The alfa chain of hemoglobin with 150 amino acids can be synthesized in just 2½ minutes. Protein synthesis in micro-organisms is even faster — a complete protein molecule can be formed in just 10 seconds.

The release of the polypeptide chain from the template seems to require a 'release factor'. The higher levels of protein structure (secondary, tertiary etc.), association of the protein with its prosthetic groups in conjugated proteins (*e.g.* globin with heme to form hemoglobin), association of enzyme proteins with the coenzymes like riboflavine and NAD, combination of more than one polypeptide chain to form a functional protein (*e.g.* A and B chains to form hemoglobin) are all brought about by the inherent nature of the polypeptide chains and does not require any further direction from the gene.

Polyribosomes actively synthesizing proteins can exist free in the cytoplasm or may be attached to membranes as in endoplasmic reticulum. The free ribosomes are responsible for synthesis of the intracellular proteins. The proteins synthesized by the polyribosomes of the endoplasmic reticulum are for export. Some may pass through the Golgi apparatus and become zymogens for secretion later.

Some animal viruses synthesize large molecules consisting of multiple proteins which are later cleaved to the functional units. In mammals some protein hormones are produced as *prohormones*. A portion of the molecule is then cleaved to give the functional hormone (*e.g.* insulin). Collagen also is synthesized as *procollagen*. Three of the molecules align themselves, followed by hydroxylation of proline and lysine and establishment of cross linkages and finally removal of amino-terminal peptides to form the collagen molecule.

Protein synthesis in the mitochondria: Mitochondria contain a few closed circular double-stranded DNA chromosomes. They have the capacity to code for a few ribosomal RNAs, tRNAs and a few proteins. The proteins are the components of the oxidative phosphorylation system. Mitochondrial DNA and function resemble those of prokaryotes and support the theory that mitochondria arose during evolution as prokaryote inclusions in the eukaryotic cell.

Energy aspects of protein biosynthesis: An equivalent of 3ATP are required for the synthesis of a peptide bond (1ATP and 2GTP). For the initiation process, one more GTP is required.

Mutation: Any change in the base sequence of one of the genes will give rise to the production of a mRNA molecule with an alteration in the base sequence. If the alteration involves the conversion of one purine base to another (A⟷G) or one pyrimidine base to another (T⟷C), it is called *'transition'*. If it involves a change of a purine base to pyrimidine and vice versa, it is called *'transversion'*.

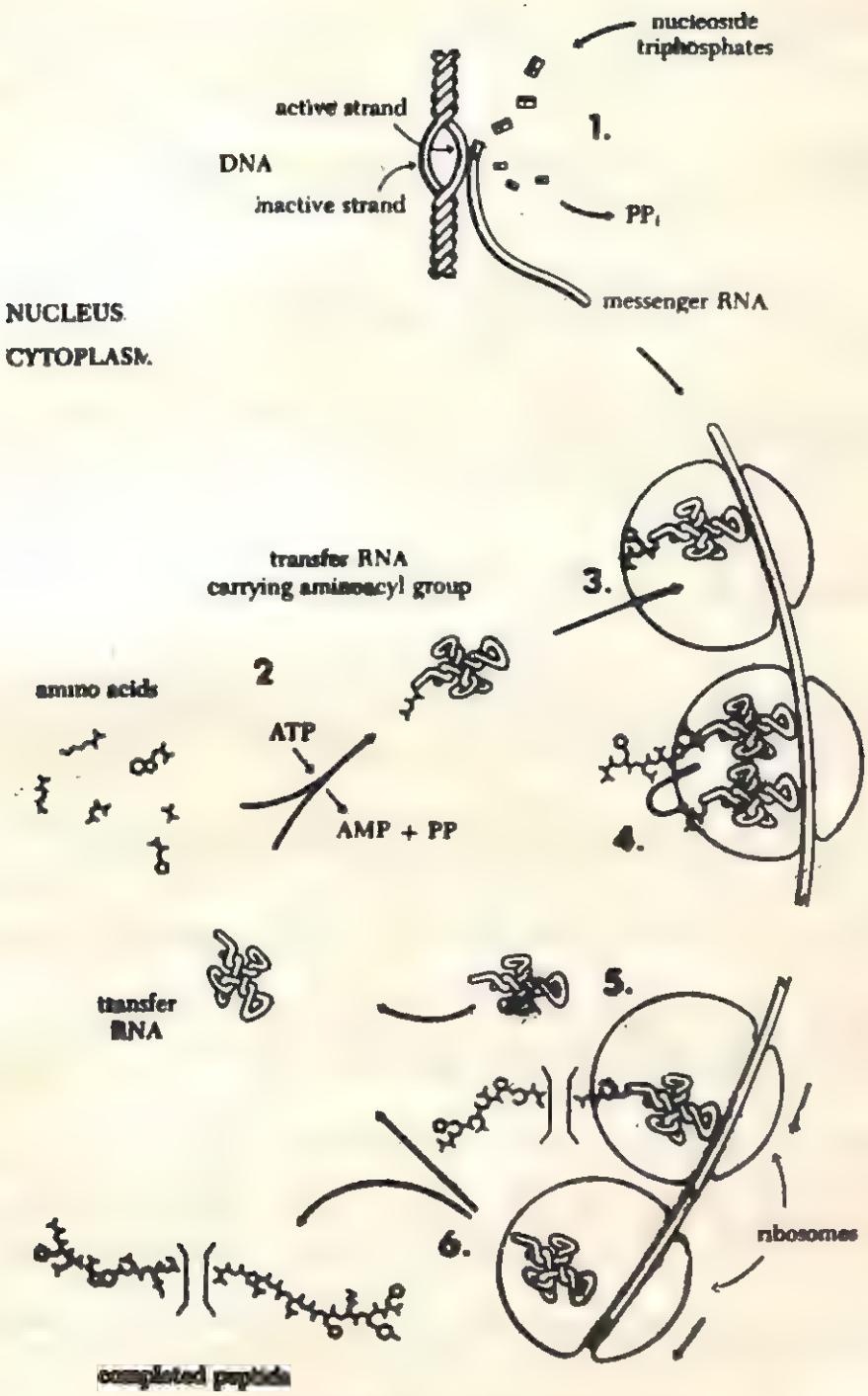


Fig. 17-2. Schematic summary of protein synthesis
Reproduced, with permission, from McGilvery, R.W.: Biochemistry, a Functional Approach; Saunders, 1970.

Schematic summary of protein synthesis. *Top. Step 1. A molecule of DNA in the nucleus unfolds, and one of its strands is used as a template to direct the formation of messenger RNA from nucleoside triphosphates, which lose inorganic pyrophosphate (PPi) as they attach to the growing RNA chain. The completed mRNA moves to the cytoplasm (bottom)*, where it binds ribosomes into a polysome, and acts as a template for protein synthesis.

The following steps are shown on separate ribosomes for clarity, but in fact they are repeated in sequence on each ribosome. The successive ribosomes grow longer and longer peptide chains as they move down the molecule of mRNA.

Step 2. Meanwhile, amino acids are combined with specific molecules of transfer RNA (tRNA) in the cytoplasm by a reaction that also involves the cleavage of adenosine triphosphate (ATP) into adenosine monophosphate (AMP) and PPi.

Step 3. The tRNA molecules, carrying the amino acids in the form of aminoacyl groups, diffuse to the polysome, where the growing peptide chain is on another molecule of tRNA already attached. The incoming tRNA, which bears the next group required for the growing peptide (in this case a leucyl residue), has the proper configuration to complex with mRNA on the ribosome.

Step 4. When the proper tRNA is in place, the peptide chain is transferred onto the amino group of the new residue brought in by tRNA, so that the chain is now one residue longer.

Step 5. When the transfer of the previous step is completed, the previously bound tRNA no longer carries a peptide chain and is free to dissociate from the ribosome, returning to the mixed pool of tRNA in the soluble cytoplasm, where it is available for transport of another molecule of its specific amino acid. The ribosome now moves along the mRNA molecule to the position where the placement of the next amino acid will be directed.

Step 6. Steps 3, 4 and 5 are repeated. As each amino acid residue adds to the peptide chain, the ribosome moves down the mRNA molecule. When a ribosome has reached the end of the molecule, the peptide is completed and is detached into the soluble cytoplasm. The ribosome itself can then move free of the mRNA and be available for attachment to the beginning of yet another molecule of mRNA (not shown).

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1. If the alteration involves the third base of a triplet, due to degeneracy of the code, it may still code for the same amino acid and no abnormality is produced (say a change of UUU to UUC of mRNA will still code for phenylalanine).

2. On the other hand, a change of the 1st or 2nd base, say GAA or GAG (for glutamic acid) to GUA or GUG will cause the insertion of valine. This is what happens in the formation of sickle cell hemoglobin (Hb-S). Normal adult hemoglobin has glutamic acid as the 6th amino acid in the B-chain, whereas in Hb-S, it is altered to valine.

3. If the alteration in the base produces a nonsensory codon, it will result in premature termination of the peptide chain and the formation of a non-functioning protein.

e.g. UCA (ser) or UUA (leu) changed to
UAA (CT) or UGA (CT).

4. Instead of alteration, if one of the bases is altogether missing, the entire code will be mis-read.

Thus UUC-CCA-ACC-GUC....
Phe -pro-thr-val-....

If C from the first triplet is missing, it becomes —
UUC-CAA-CCG-UC....
phe gln-pro-....

An altogether different protein is formed.

5. If an entire triplet is missing, a protein with one amino acid missing is formed.

6. Addition of a nucleotide will also result in complete alteration of the amino acids as in (4) above.

7. Addition of a triplet will result in introduction of a new amino acid.

Many of these abnormalities are actually met with in hemoglobinopathies.

The effect of ultraviolet radiation is to cause the formation of dimers between bases of the two DNA strands in the helix (usually between two thymines) thereby making the strands inseparable in that region and hence not available for replication or transcription.

Nitrogen-mustards can cause cross-linkages between guanine residues in the two DNA strands with similar results.

Treatment of a nucleic acid with nitrous acid deaminates the bases and changes cytosine to uracil and adenine to inosine (read as guanine in the code), and thus causes mutation.

Acridine dyes like proflavine and alkylating agents can also alter the DNA bases and cause mutations.

The Regulation of the Gene Activity:

All genes are present in every cell of the organism during its entire life span. Yet they exert their influence to different extent in different cells of various tissues and organs. Even in the same cells of the same tissue or organ, the influence is exerted to a varying extent from time to time.

The ovum and spermatozoa from which the complex organism arises must necessarily contain genes required for the synthesis of all the proteins that can be synthesized by all the different cells in the organism. In the human sperm or egg cell, the DNA is just about

6 picograms (6×10^{-12} g) and yet it carries all the information for all the thousands of characteristics of the human. But in the developed organism, only the reticulocytes retain the ability to form hemoglobin and only the beta cells of the pancreas retain the ability to synthesize insulin. In all other cells, the genes concerned with the above functions are somehow permanently suppressed.

On the other hand, if a culture of E. Coli is grown on a medium containing tryptophan, the production of enzymes concerned with the biosynthesis of tryptophan is suppressed in that organism. This is only a temporary induced phenomenon of a negative effect on gene function.

Another phenomenon observed is the induction of certain enzymes in the presence of their substrate. The enzymes concerned in the metabolism of lactose are produced by E. Coli only when lactose is present in the culture medium. This is a positive effect on gene function.

The gene activity seems to be regulated in two different ways:

1. Blocking of gene activity on a long standing basis as in case of hemoglobin and insulin synthesis. The mechanism for this is poorly understood. The proteins of the nuclei are probably responsible. The basic proteins, histones, probably help in maintaining the correct packing and positioning of nuclear DNA. The acidic proteins of the nucleus are probably responsible for binding to the non-functional genes and preventing their activity.

2. Blocking off and on depending on immediate metabolic needs:

(a) Enzyme induction: The entry of a substrate into the cell induces the formation of the enzymes required for its metabolism. In the E. Coli grown on a lactose containing medium, three enzymes concerned with the hydrolysis and further metabolism of lactose are produced in abundant quantities. This is known as enzyme induction.

(b) Enzyme Repression: On the other hand, E. Coli grown on a tryptophan-free medium elaborate five enzymes concerned with the biosynthesis of tryptophan; if tryptophan is now added to the medium, the E. Coli rapidly lose the ability to synthesize those enzymes. This is enzyme repression.

Jacob and Monod (1961) have studied the phenomena and postulated a possible mechanism to explain them. This is known as the "OPERON" concept.

The block in the production of the enzyme protein (or removal of such block) seems to occur at the "transcription" stage, *i.e.*, in the formation of mRNA on the DNA template. The genes concerned in the synthesis of proteins with related function are located sequentially as a single unit in one region of the chromosome. The unit is called the

OPERON. We have thus a *lac* operon for the metabolism of lactose and a *trp* operon for the biosynthesis of tryptophan. At the beginning of each operon unit, there is an *operator gene* whose function is only to regulate the activity of the other genes in the unit. This is followed by *structural genes*, as many in number as the number of proteins to be synthesized. These are directly concerned in the synthesis of mRNA. The mRNA for all the functional proteins of the unit is formed as one strand and is called the *polycistronic mRNA*. At a remote portion of the chromosome is located another gene called the *regulator gene*. This has a controlling effect on the operator gene. It synthesizes large protein molecules called *repressors* which combine with and block the function of the operator gene, which in turn will result in blocking the function of all the structural genes in the operon unit.

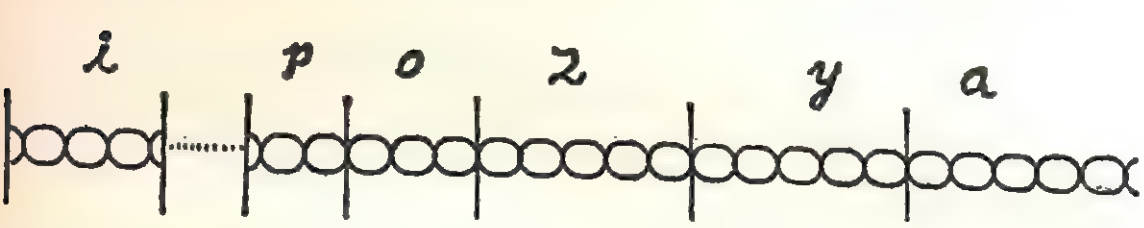
An enzyme inducer (like lactose or even its analogue, isopropylthiogalactoside or IPTG) can itself combine with the repressor molecule and thus prevent its combination with the operator gene. The operator gene and the structural genes hence become functional and mRNA for the functional proteins is synthesized. A *lac* repressor had been actually isolated from E. Coli. It is a protein with a molecular weight of 150,000.

Trp Operon: In addition to the promoter and operator genes, there is also a second regulatory site — the attenuator. This is located just preceding the structural genes (which are five in number and produce the five enzymes required for tryptophan synthesis). If there is an abundance of tryptophan and all the available $tRNA^{Trp}$ is saturated, the attenuator inhibits further production of mRNA from the structural genes. If, on the other hand, there is much of free $tRNA^{Trp}$ uncharged with tryptophan, the synthesis of mRNA from the structural genes proceeds rapidly.

Induction of gene expression by steroid hormones: The steroid hormones passively diffuse through the plasma membrane and associate with a specific, soluble receptor protein. The steroid-receptor complex enters the nucleus and binds to specific sites on the chromatin and exerts a controlling effect (inhibitory or stimulating) on the formation of mRNA by the particular genes.

Cyclic AMP promotes the initiation of transcription of a number of inducible operons. It does so by forming a complex with a specific protein and the complex will bind to the promoter site in the operon and stimulate the initiation of transcription. High levels of glucose in the medium decrease cyclic AMP levels in the cell and thus decrease the synthesis of several catabolic enzymes.

A schematic diagram of the *lac* operon is shown below:



i = regulatory gene

p = promoter site. The enzyme, RNA-polymerase, binds here.

o = operator gene.

z = the structural gene for beta-galactosidase enzyme protein.

y = the gene responsible for the permeation of galactose into the cell.

a = the gene for galactoside acetylase enzyme protein. The function of this enzyme is not clear.

Inhibitors of protein synthesis:

Mitomycin: It causes the formation of covalently bonded cross links between the two DNA strands and prevents their separation. Hence replication of DNA strands cannot occur.

Actinomycin-D: This binds itself to the surface of DNA and prevents the formation of mRNA.

Tetracycline, streptomycin and chloramphenicol: They selectively combine with one or more ribosome components (30S or 50S) and prevent the combination with aminoacyl-tRNA at the initiator site or the combination between the two ribosomes.

Puromycin It has a structural resemblance to the binding site of tRNA. Hence a growing polypeptide chain on the preceding tRNA may get transferred on to puromycin, but subsequent steps do not occur. Polypeptide chains hence remain incomplete.

Cycloheximide: It inhibits the formation of the peptide bond by inhibiting the enzyme 'peptide synthetase'.

Rifamycin: It is a specific inhibitor of DNA-dependent RNA-polymerase.

Interferons: These are small glycoproteins (M.W. 26,000 - 38,000) and may be derived from leukocytes or fibroblasts. Cells exposed to interferon develop an antiviral state. The replication of several viruses is inhibited. Cell growth also may be inhibited. One

mechanism of action is by inhibition of viral mRNA translation at the initiation stage by phosphorylating and thereby inactivating the initiation factors, eIF-2 (compare with the actions of adenylate cyclase). Another mechanism is by stimulating the synthesis of a trinucleotide of adenylic acid. The three nucleotides are linked by a 2',5' linkage (instead of the usual 3',5' linkage). This unusual trinucleotide activates an endogenous ribonuclease which causes premature degradation of the mRNA.

Diptheria toxin: It inhibits translation by blocking the elongation phase of protein synthesis by its effect on elongation factor, EF-2.

Synthesis and interconversion of amino acids:

Several of the amino acids can be synthesized in the body from the alfa keto acids formed during carbohydrate metabolism by substituting the keto group by an amino group. This process is discussed in detail under transamination in the succeeding paragraphs. By this process alanine, aspartic acid and glutamic acid can be formed directly by amination of pyruvic acid, oxaloacetic acid and α -ketoglutaric acid. Glycine, serine, cysteine, cystine, proline, hydroxyproline, ornithine and citrulline can be formed indirectly from the three amino acids originally formed. Tyrosine can be formed from phenylalanine. Since these can be readily synthesized in the body even if they are not present in food, they are said to be 'non-essential amino acids.'

Essential amino acids: Threonine, valine, leucine, isoleucine, lysine, methionine, phenylalanine, tryptophan, arginine and histidine cannot be synthesized in the body. They are still essential as constituents of tissue proteins and hence require to be supplied in food. They are called the 'essential amino acids'. In their deficiency, protein synthesis does not occur and the nitrogen excretion is more than the intake (negative nitrogen balance) leading to wasting of the tissues and severe symptoms. There is, possibly, synthesis of histidine and arginine to a small extent in the human. Hence these two amino acids can be dispensed with for short periods in the adult and are not absolutely essential. But during growth and convalescence, they become absolutely essential due to the increased demands for protein synthesis. Much of the requirement of methionine and phenylalanine is for the synthesis of cysteine and tyrosine. If there is abundance of cysteine and tyrosine in the diets, the requirement of methionine and phenylalanine will be correspondingly less.

Origin of Life

Geologists estimate that life arose three or four billion years ago. The earth's atmosphere then did not contain any oxygen. It contained reducing gases like hydrogen, methane and other hydrocarbons, hydrogen cyanide, ammonia and hydrogen sulfide. There was only one tenth of the quantity of water now present. Electrical discharges in such an atomosphere can produce monosaccharides, amino acids and peptides, and purine and pyrimidine bases. From these building blocks the higher molecular weight compounds might have arisen. Nucleic acid molecules, if formed, can, in the presence of polypeptides, multiply autocatalytically. The genetic code itself would have evolved quite early, since the code is universal to all living organisms.

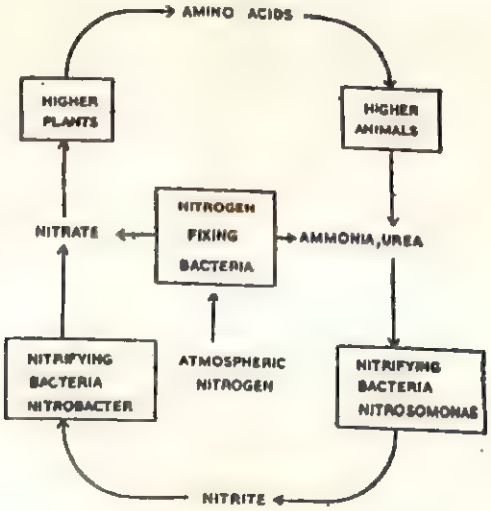
Oxygen had come into the atmosphere as a result of photosynthetic reactions in early plant life and by hydrolysis of water.

The earliest structures formed might be of the nature of coacervates — droplet-like aggregates that form spontaneously in aqueous solutions of two or more different colloids. The interior of such droplet often gets enriched in certain substances from the environment. This might explain the formation of the 'milieu interior.' The surface of the coacervates presents membrane-like structures closely resembling the membranes of the living cells.

Symbiosis and interspecific gene transfer:
It is possible that some of the organelles of the eukaryote cells might have arisen as inclusions during early symbiotic existence with unicellular organisms. e.g. chloroplasts of higher plants from the blue-green algae and mitochondria of animal cells from aerobic bacteria.

Nitrogen Fixation and Assimilation in Microorganisms:
Microorganisms in the soil can take up atmospheric nitrogen and form ammonia and nitrates. These will serve as the source material for plants which can produce ammonia from the nitrites and nitrates and incorporate it into proteins.

Bacteria like Rhodospirillum rubrum, Azotobacter and Clostridium pasteurannum and algae like Nostoc muscorum are particularly active in forming ammonia and nitrates from atmospheric nitrogen. $N_2 + 3H_2 \longrightarrow 2\,NH_3$



THE NITROGEN CYCLE

Fig. 17.3

The reduction of nitrogen to ammonia is brought by a 'nitrogenase' system which contains an iron-protein and a flavoprotein and requires Mg^{++} ions and energy from the breakdown of ATP.

Nitrosomonas bacteria can oxidize ammonia to nitrite. The nitrite is further oxidized to nitrate by the Nitrobacter. The bacteria Rhizobium can fix up nitrogen into nitrates symbiotically in the root nodules of leguminous plants.

Plants and other microorganisms take up the nitrites and nitrates and utilize them after first reducing them to ammonia by the enzymes 'nitrate and nitrite reductases.' Blue-green algae, the prokaryotes found in the soil, fresh water and oceans, are the most self-sufficient cells. They obtain their energy from sunlight, their carbon from CO_2 their nitrogen from atmosphere, and their electrons from H_2O. They are considered the earliest forms of life in evolution.

OXIDATION OF AMINO ACIDS

Amino acids differ from carbohydrates and lipids in containing a nitrogenous moiety (amino group) which is not completely oxidized in the body. The amino group is transferred to an alfa keto acid to synthesize some of the nonessential amino acids or is removed and converted to an excretory product, urea, by processes known as transamination and oxidative deamination. The remaining portion of the amino acid containing only carbon, hydrogen and oxygen, called the 'carbon skeleton', is oxidized like the carbohydrates and the lipids. The intermediate compounds formed during the oxidation of most amino acids resemble those of carbohydrate metabolism and may be converted to glucose by 'gluconeogenesis.' These amino acids are called 'glycogenic amino acids.' Others form acetate or acetoacetate, the intermediates formed during fatty acid metabolism. They are called the ketogenic amino acids. They are listed in Table 17.2.

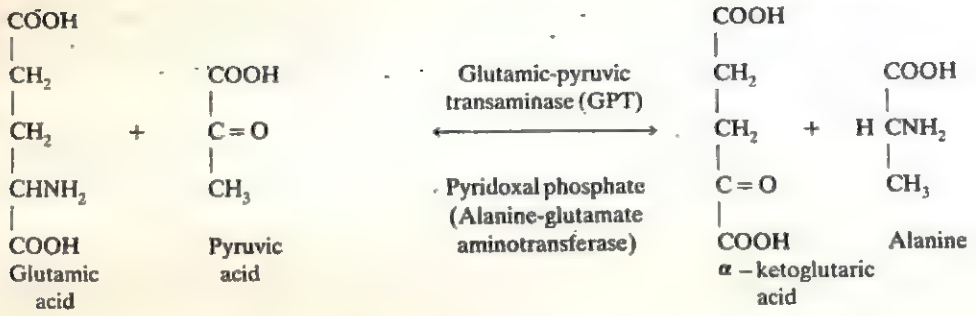
TABLE 17.2

Glycogenic	*Ketogenic*	*Glycogenic and ketogenic*
glycine, alanine, serine, threonine, valine, aspartic and glutamic acids, cysteine, cystine and methionine, proline and hydroxyproline, arginine, histidine.	leucine	isoleucine, lysine, phenylalanine, tyrosine, tryptophan.

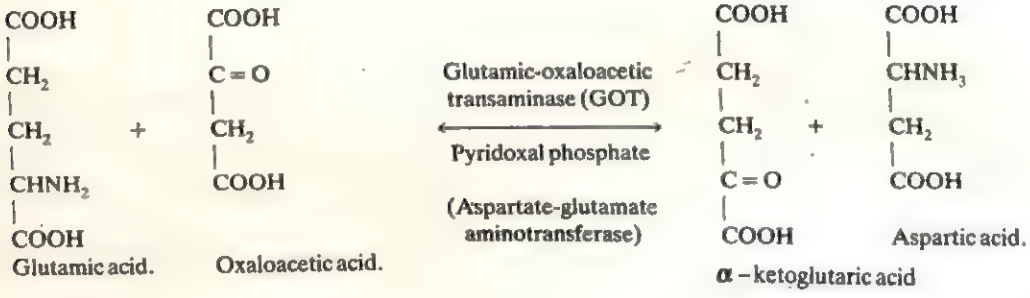
The general reactions applicable to all amino acids are discussed below. Metabolism of individual amino acids follows.

Transamination: Enzymes called 'transaminases' or 'aminotransferases' catalyze the transfer of the amino group of an amino acid to an alfa keto acid to form a new amino acid and a new keto acid.

Aminotransferases are present in the liver, kidney and the brain.



Instead of pyruvic acid, if oxaloacetate is the keto acid, the new amino acid formed is aspartic acid.



The reaction is not confined only to the formation of alanine and aspartic acid. Several other keto acids can be transaminated to form their corresponding amino acids. Pyridoxal phosphate acts as a coenzyme in these reactions by its reversible conversion to pyridoxamine phosphate.

Serum contains both glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase enzymes. It is further found that serum glutamic-oxaloacetic transaminase (SGOT) is markedly elevated in cardiac infarction. Serum glutamic-pyruvic transaminase (SGPT) levels are increased in hepatic disease.

Lysine, threonine, proline and hydroxyproline are the amino acids which cannot be aminated from their respective keto acids.

Transamidation: The amide nitrogen of glutamine can be transferred to a keto group in a manner similar to transamination. If transferred to an α—keto acid, an amino acid can be formed. If transferred to the keto group of fructose, glucosamine is formed. The reactions of transamination and transamidation serve in the interconversion of amino acids and synthesis of the non-essential amino acids. All tissues have varying capacities for transamination and transamidation. The liver is very active in both and serves a central role in amino acid metabolism.

Oxidative deamination of amino acids: This is the mechanism by which amino group is removed from the alfa carbon to form a keto group and free ammonia. Liver, kideny and several other tissues contain D- and L-amino acid oxidases, active in deamination of the D-or L-amino acids as the case be. But most of the deamination occurs in liver normally. The enzymes contain FAD or FMN as the prosthetic group. The deamination proceeds as follows:

$$\underset{\text{Amino acid}}{R\,CH\,NH_2\,COOH} + FP \xrightarrow{\text{L-amino acid oxidase}} \underset{\text{Imino acid}}{RC=NH\,COOH} + FPH_2$$

$$\underset{\text{Imino acid}}{RC=NH\,COOH} + H_2O \xrightarrow{\text{Spontaneous}} \underset{\text{}\alpha\text{-Keto acid}}{R\,CO\,COOH} + NH_3$$

$$FPH_2 + O_2 \longrightarrow F.P + H_2O_2$$

$$H_2O_2 \longrightarrow H_2O + \tfrac{1}{2}\,O_2$$

L-Glutamic acid is an exception in that it is deaminated by an enzyme L-glutamic acid dehydrogenase which requires NAD or NADP as coenzyme. The deamination is reversible in this case, whereas the deamination by L-amino acid oxidase is not reversible. The L-amino acid oxidase levels of most tissues are very low. In fact most amino acids are synthesized and deaminated by transamination reactions with glutamic acid which alone is capable of taking up ammonia direct while in the form of alfa-ketoglutarate.

Glycine is another amino acid which is acted upon by a specific enzyme 'glycine oxidase.' The amino acids serine and threonine which already have one 'OH' in their molecule are deaminated nonoxidatively by enzymes known as dehydrases.

$$\underset{\text{Glycine}}{\tfrac{1}{2}\,O_2 + CH_2\,NH_2\,COOH} \xrightarrow{\text{Glycine oxidase}} \underset{\text{Glyoxylic acid}}{CHO.COOH} + NH_3$$

$$\underset{\text{Serine}}{CH_2OH.CHNH_2\,COOH} \xrightarrow{\text{Serine dehydrase}} \underset{\text{Imino acid}}{CH_3.C=NH.COOH} + H_2O$$

$$CH_3\,C=NH.COOH + H_2O \longrightarrow \underset{\text{Pyruvic acid}}{CH_3\,CO\,COOH} + NH_3$$

Decarboxylation: Tissues like liver and the microorganisms of the intestinal tract contain enzymes called 'decarboxylases' which require pyridoxal phosphate as coenzyme. They remove CO_2 from the carboxylic group and convert the amino acid to its corresponding amine. This is mostly a process confined to putrefaction in intestines and produces toxic amines like tyramine, tryptamine, putrescine and cadaverine from tyrosine, trytophan, ornithine and lysine.

$$R.CH\,NH_2\,COOH \xrightarrow[\text{Pyridoxal phosphate}]{\text{decarboxylase}} R\,CH_2\,NH_2 + CO_2$$

Some of the biologically important amines formed by decarboxylation of amino acids are listed below:

Amino acid	*Amine*	*Occurrence and significance*
Lysine	cadaverine	product of putrefaction
Ornithine	putrescine	product of putrefaction
Methionine	spermidine	ribosomes and sperm
Arginine	agmatine	putrefaction
Serine	ethanolamine	phosphatides
Threonine	propanolamine	vitamin B_{12}
Cysteine	beta-mercapto ethanolamine	coenzyme-A
Aspartic acid	beta-alanine	coenzyme-A
Glutamic acid	gamma-amino butyric acid (GABA)	brain (ganglion inhibitor)
Histidine	histamine	effects blood pressure
Tyrosine	tyramine	contracts uterus
3, 4-Dihydroxy -phenylalanine	dopamine	precursor of adrenaline and noradrenaline
Tryptophan	tryptamine	5-hydroxytryptamine is serotonin.

Metabolism of the carbon skeleton: After removal of the ammonia, the alfa keto acid can follow one of three pathways.

(a) Reamination to form original amino acid.

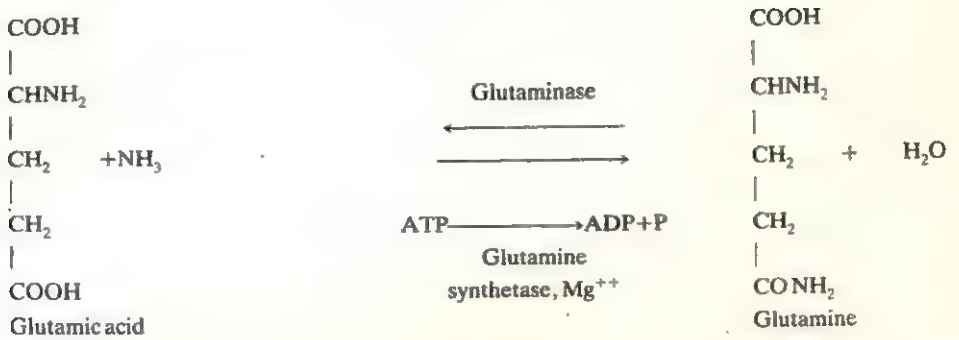
(b) Oxidation to CO_2 and H_2O.

(c) Formation of glucose (glucogenic amino acids), or ketone bodies (ketogenic amino acids). Further details are dealt in the metabolism of individual amino acids.

Metabolism of ammonia: The ammonia liberated by deamination reactions is not allowed to accumulate but rapidly metabolized with the result that very little escapes into the blood. Blood ammonia is normally 10-12μg/100 ml. Increase in blood ammonia is highly toxic to the central nervous system and may be fatal. Liver is the main site of ammonia metabolism. Ammonia production also occurs in the liver. Some ammonia is produced also in the intestines due to bacterial action on food amino acids and may be absorbed. In diseases involving liver, there may be increase in blood ammonia levels with depression of the C.N.S., a condition called hepatic coma.

Methods available for removal of ammonia:

1. Amination of α-keto acids to form amino acids.
2. Amidation of glutamic acid to form glutamine.



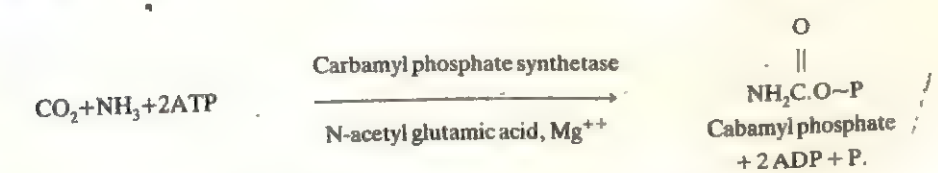
The glutamine enters the blood stream and is transported to the kideny, where the reverse reaction is brought about by the enzyme glutaminase. The ammonia so liberated is utilized to replace sodium and potassium of glomerular filtrate and is excreted as ammonium chloride or phosphate. Formation of glutamine is the only mechanism for removal of NH_3 formed in brain.

3. Formation of urea in the liver. Quantitatively this is the most important method for metabolism of ammonia and will be considered in detail now.

UREA FORMATION: KREBS-HENSELEIT CYCLE

The steps in the synthesis of urea in the liver were elucidated by Krebs and Henseleit. The cycle is named after them. There is ample evidence to show that this synthesis occurs in the liver. If the kidenys are removed in an experimental animal, there is a sharp rise in the blood urea levels. This can be prevented if the liver also is removed. In cirrhosis of the liver, a disease where the functioning of the liver is much below normal, blood urea levels decrease with a simultaneous increase of ammonia. Similar results are seen where the liver is excluded from circulation by an anastomosis between the portal vein and vena cava (porto-caval shunt). Enzyme studies have revealed that the enzyme 'arginase' which is required in the final step of producing urea from arginine is present only in the liver and absent in all other tissues.

1. *Formation of carbamyl phosphate:* This is the first step in the urea cycle. Carbondioxide is first activated and the active CO_2 now combines with NH_3 to form carbamyl phosphate. Both reactions require energy from ATP and the presence of N-acetyl glutamate (AGA) and are brought about by the enzyme carbamyl phosphate synthetase.



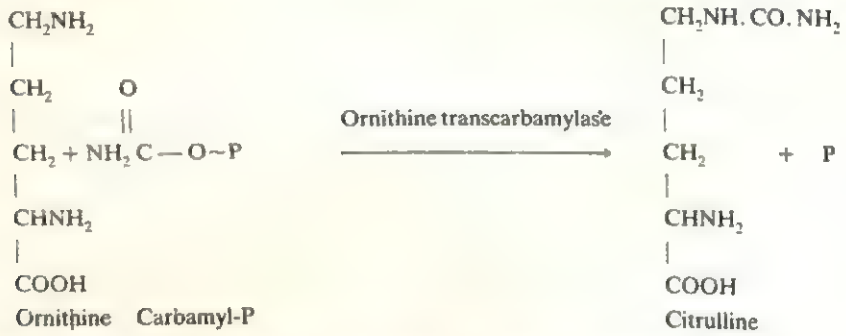
The urea cycle is effectively controlled by N-acetylglutamic acid. It is formed by a combination of acetyl-CoA and glutamic acid.

Glutamate + acetyl-CoA ⟶ nAGA + HS-CoA

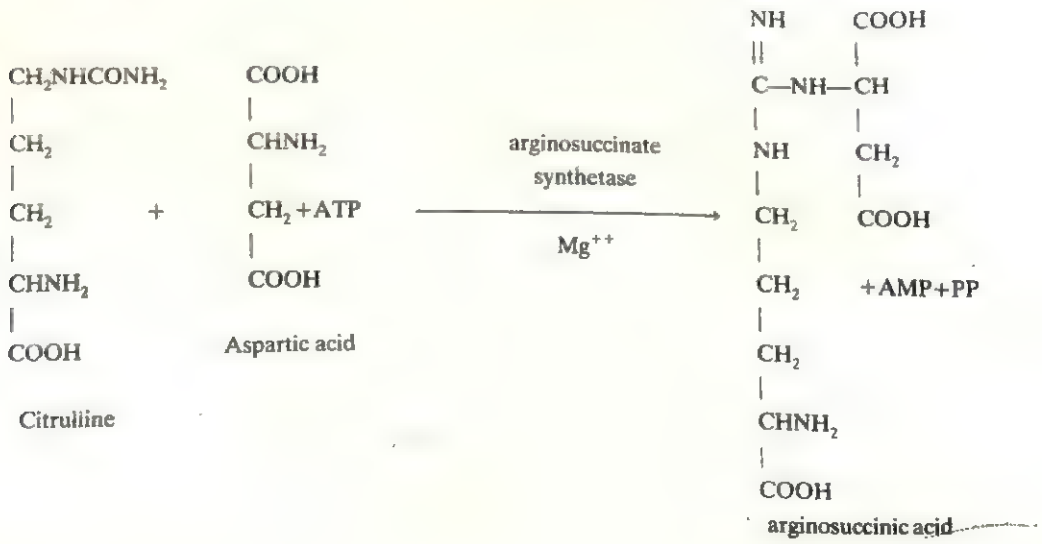
When the amino acid levels are high, there is increased formation of nAGA which allosterically stimulates carbamyl phosphate synthase (carbamyl phosphate synthase I in this case) and drives the urea cycle forwards.

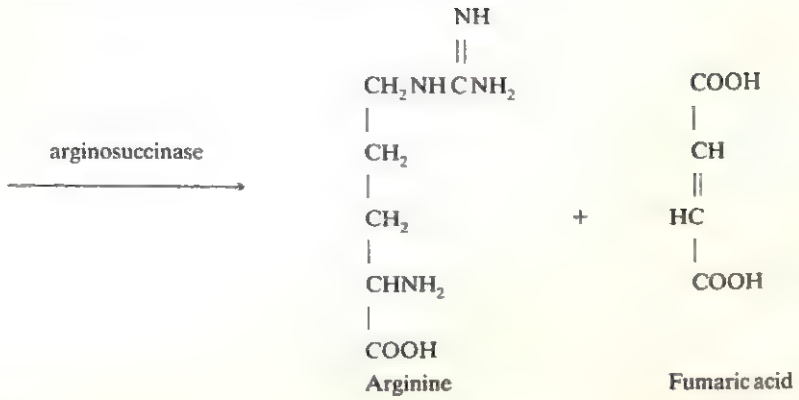
Glutamate dehydrogenase also plays an important role. When glutamate, aspartate, glutamine and aspargine are available in plenty, the enzyme acts in the direction of deamination. When these amino acids are in short supply, the enzyme functions in the direction of amination.

2. *Formation of citrulline from ornithine:* This is catalyzed by the enzyme ornithine transcarbamylase.



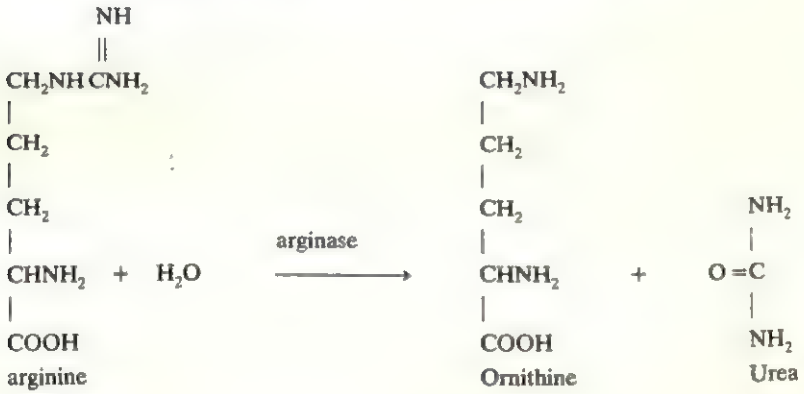
3. *Formation of arginine from citrulline:* This is facilitated by condensation of citrulline with aspartic acid to form arginosuccinic acid which then splits into two molecules – arginine and fumaric acid.





The fumaric acid can be converted to malic acid and from that to oxaloacetate in the citric acid cycle. The oxaloacetate on amination forms aspartic acid again.

4. *Formation of urea from arginine:* The enzyme, arginase, which is present only in the liver, will now hydrolyze arginine to form urea and ornithine.



Ornithine regenerated in step 4 can again participate in the cycle by entering at step. (2). Aspartic acid has lost its ammonia in step (3) in converting the ureido group of citrulline to the guanido group of arginine. But it can be reformed by running through citric acid cycle and later getting aminated.

The formation of carbamyl phosphate and the transfer of carbamyl group to ornithine to form citrulline occur in the mitochondrial matrix. The citrulline formed leaves the mitochondria and the remaining steps upto the formation of urea and ornithine occur in the cytosol. The ornithine now reenters the mitochondrial matrix to repeat the cycle again.

Thus carbamyl phosphate contributes the carbon and one of the aminonitrogens of urea. The second amino nitrogen is derived from the alfa amino group of aspartic acid. The reactions are represented schematically in Fig. 17-4.

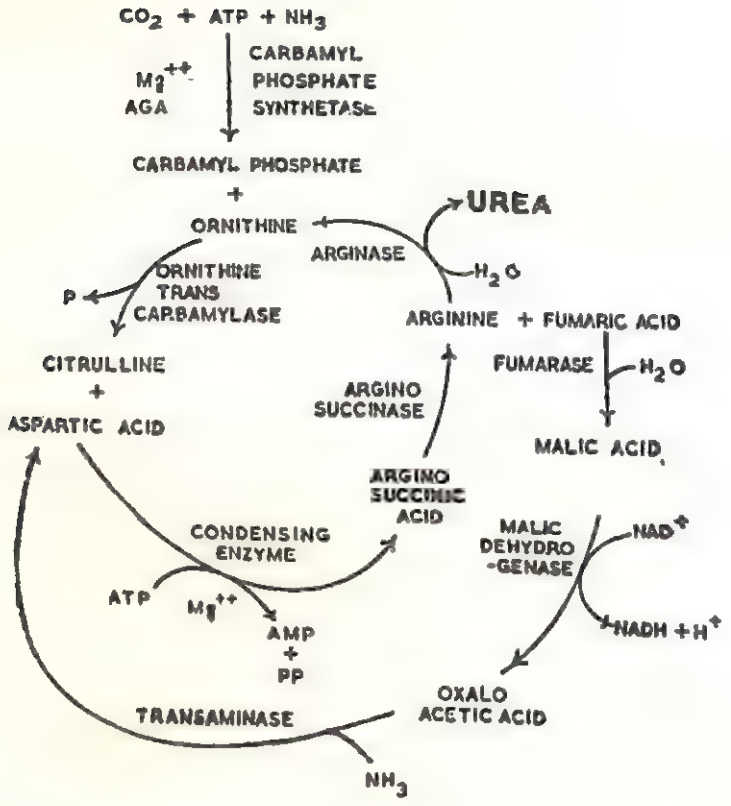


Fig. 17-4. Urea formation (Krebs-Henseleitt urea cycle)

Defeciency may occur of any of the 5 enzymes concerned in urea synthesis. Deficiency of cárbamyl phosphate synthetase and ornithine transcarbamylase will result in accumulation of ammonia in blood (hyperammonemia, Types I and II). Deficiency of arginosuccinate synthetase causes citrullinemia. Deficiency of arginosuccinase results in accumulation of arginosuccinate in blood and arginosuccinicaciduria. Deficiency of arginase causes hyperarginemia.

Blood urea: Normal individual has a blood urea of 15 to 40 mg/100 ml. Urea is mainly excreted by the kidney. It is freely permeable through the glomerulus but a portion is reabsorbed by the renal tubule and the rest excreted. The urea clearance value is about 75 ml/min (maximal clearance *i.e.*, when urine formation is 2.0 ml/min or more). When urine formation is below 2.0 ml/min., the clearance rate is only about 54 ml/min (standard clearance).

Energy production in amino acid oxidation:

The energy production can be calculated for glutamic acid oxidation thus:

1.	*Glutamic acid* ⟶ alfa-ketoglutaric acid: $NADH+H^+$ is produced which on oxidation through respiratory chain yields	3 ATP
2.	*α-Ketoglutarate to succinyl CoA:* $NADH+H^+$ oxidation	3 ATP
3.	*Succinyl CoA* ⟶ succinate: substrate phosphorylation	1 ATP
4.	*Succinate to fumarate:* FP. H_2 oxidation	2 ATP
5.	*(Fumarate to malate) malate to oxaloacetate:* $NADH+H^+$ (oxaloacetate ⟶ pyruvate $+CO_2$)	3 ATP
6.	Pyruvate ⟶ acetyl CoA ⟶ CO_2 and H_2O	15 ATP
		27 ATP

2 ATP are required for the formation of carbamyl phosphate. One ATP is split to AMP and pyrophosphate (loss of two energy-rich phosphates) in the synthesis of arginosuccinate. Thus a total of 4 ATP are expended for each molecule of urea synthesized. Since glutamic acid contributes only one of the two nigtrogens of urea, the energy expenditure for glutamci acid metabolism is 4/2 *i.e.* 2 ATP.

Balance 25 ATP

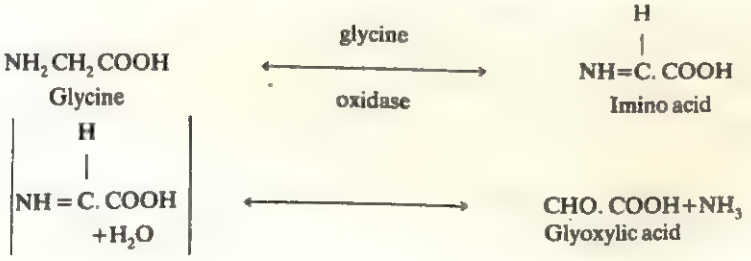
Thus 25 ATP are produced in the oxidation of one molecule of glutamic acid.

METABOLISM OF INDIVIDUAL AMINO ACIDS

Glycine

It is a non-essential, glycogenic amino acid.

Glycine can be synthesized from CO_2 and NH_3 by *glycine synthase.* Pyridoxal phosphate is required as coenzyme. Tetrahydrofolate contributes a methylene group.

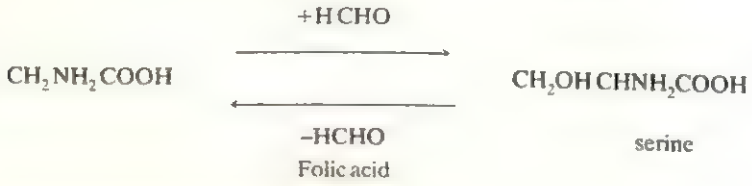


The glyoxylic acid can be further oxidized to oxalate or decarboxylated to form formate.

Though a non-essential amino acid, it serves very important metabolic functions:

1. Along with succinate, it takes part in heme synthesis. The nitrogen of each of the pyrrole rings, a carbon adjoining the nitrogen and also the carbon of the methylene bridge are all derived from glycine.

2. Glycine can form serine by taking on a formate molecule.



This is a readily reversible reaction.

3. The formate formed from glycine can be taken up by tetrahydrofolic acid and take part in all reactions involving a one carbon fragment.
4. Glycine moiety is incorporated into purine ring in positions C_4, C_5, and N_7.
5. Glycine is one of the three amino acids required for formation of creatine.
6. It is also a constituent of glutathione.
7. It takes part in detoxicating mechanisms.
   Benzoic acid+Glycine ⟶ Hippuric acid.

Abnormalities in glycine metabolism:

1. *Primary hyperoxaluria:* due to diversion of more glycine to oxalate formation rather than formate production. Urine contains large amounts of oxalates.

2. *Glycinuria:* There is a decreased tubular reabsorption of glycine in the kidney.

Alanine

It also is a non-essential, glycogenic amino acid. Deamination or transamination produces pyruvic acid which can be readily converted to glucose or oxidized in citric acid cycle. *β*-alanine is a constituent of pantothenic acid.

Glucose-Alanine cycle

Glucose is released from liver by glycogenolysis and gluconeogenesis during muscular contraction. Glucose is utilized by muscle by glycolysis, producing pyruvate. While part of this pyruvate is converted to lactate, the rest is aminated to form alanine. Both are returned

to the liver and can participate in gluconeogenesis, to form fresh glucose. Formation of alanine from pyruvate in muscle also helps in removing some of the NH_3 formed in that tissue during amino acid metabolism. The cycle of transport of glucose from liver to muscle and of alanine from muscle to liver is known as glucose–alanine cycle.

Serine

This is also a non-essential, glycogenic amino acid. By the action of dehydrase on serine, pyruvic acid is produced. The β-carbon of serine serves as a source for one carbon fragment.

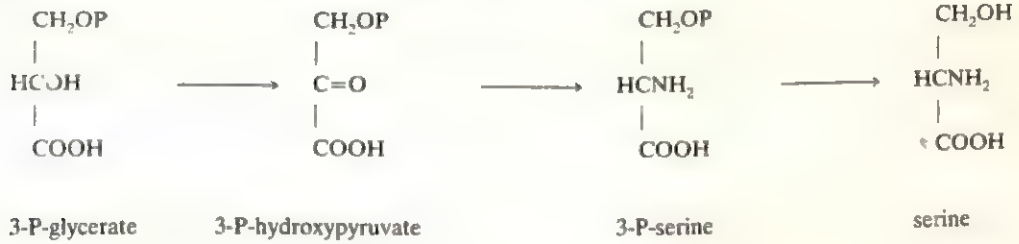
$$\underset{\text{Serine}}{CH_2OH\,CHNH_2\,COOH} \longrightarrow HCHO + \underset{\text{glycine}}{CH_2NH_2COOH}$$

(formate)

The hydroxy group of serine is esterified with phosphate in the phosphoproteins.

Serine is also a constituent of a group of phospholipids known as cephalins.

Serine can be synthesized in the mammalian tissues from 3-phosphoglycerate which is oxidized to 3-phosphohydroxypyruvate. This is now transaminated to form phosphoserine. Phosphate is then removed by hydrolysis to form serine.



Threonine

This is an essential amino acid. It is glycogenic. It is one of the amino acids which cannot be formed by transamination of the keto acid. It is broken down by the enzyme 'threonine aldolase' into acetaldehyde and glycine. The acetaldehyde is converted to acetate. Glycine is metabolized as already described.

In the vertebrates, threonine is converted by a dehydratase enzyme (similar to serine dehydratase) to from α-ketobutyric acid, which, on oxidative decarboxylation, gives propionyl-CoA.

$$CH_3CHOHCHNH_2\,COOH \longrightarrow CH_3\,CH_2\,COCOOH \longrightarrow CH_3CH_2\,CH_2\,COSCoA$$

$\longrightarrow$ Methylmalonyl CoA $\longrightarrow$ Succinyl CoA $\longrightarrow$ Gluconeogenesis.

Another pathway is by formation of 2-ketopropanol ($CH_3COCH_2\,OH$) which, can be converted to pyruvate.

Both are glycogenic pathways.

It is a constituent of all proteins and, like serine, it holds the phosphate in case of phosphoproteins.

BRANCHED CHAIN AMINO ACIDS

Valine, Leucine and Isoleucine

All the three are essential amino acids. The corresponding keto acids can be aminated to form them. Hence the keto acids can substitute for them in diet. Valine is glycogenic. On deamination, it forms methylamlonyl-CoA which is then converted to succinyl-CoA.

Leucine, on the other hand,forms one molecule acetoacetate and one of acetate. They can be considered as one and a half ketone bodies. Thus it is the most potent of ketogenic amino acids.

Isoleucine, after oxidative removal of one carbon, forms one molecule of propionic acid and one of acetate (½ ketone body). The propionate is converted through methylmalonate to succinate. Thus it is glycogenic and weakly ketogenic.

Branched chain amino acids are avidly taken up by brain and utilized. These are supplied by absorption from the gastrointestinal tract following a meal and are preferentially taken up by the brain and muscle. In the postabsorptive state, they are supplied by protein breakdown in the muscle.

The metabolism of leucine is outlined in fig. 17-5 as an example of the pathways typical of these amino acids. Step (2) in which the alfa keto acid is converted by oxidative decarboxylation to the corresponding -CoA derivative (with one carbon less) is similar to oxidative decarboxylation of alfa keto glutarate and pyruvate. The enzyme complex requires TPP, lipoate, FAD, NAD and coenzyme-A as cofactors. The enzyme is called *'branched chain 2-keto acid dehydrogenase complex'*. A deficiency of the enzyme causes maple syrup urine disease.

Maple syrup urine disease: A metabolic defect involving the branched chain amino acids will result in the excretion of their keto acids in urine. The defect is in step (2), the oxidative decarboxylation of the alfa keto acids. The urine is said to have the odor of maple syrup.

There is rapid deterioration in the first two months of life. If the child survives, he manifests serious mental retardation.

Hypervalinemia: There is an accumulation of valine. Leucine and isoleucine are metabolized normally. This is due to a defect in the specific aminotransferase for valine.

There appears to be a common aminotransferase for leucine and isoleucine. Both amino acids accumulate in its defect or deficiency.

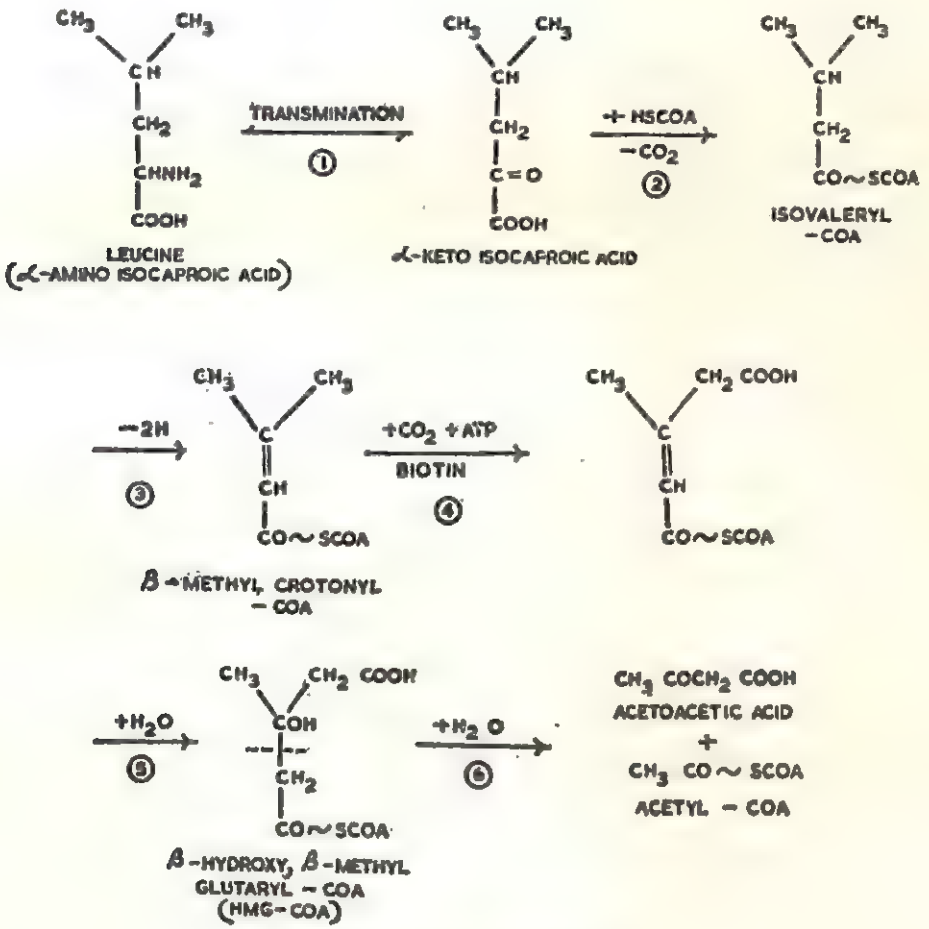


Fig. 17-5. Metabolism of leucine.

Lysine

This is an essential amino acid and is glycogenic and ketogenic. It is not present in adequate amounts in cereal proteins. Hence its deficiency in vegetarian diets is quite common. This is another of the amino acids which cannot be aminated from its keto acid.

L-Lysine condenses with a molecule of alfa-ketoglutarate to form a Schiff base which is then reduced to 'saccharopine'. This undergoes dehydrogenation followed by hydration, whereby it splits into a molecule of glutamate and a semialdehyde of alfa-aminoadipate. This amounts to a deamination of the eta-amino group. Alfa-aminoadipate now transaminates

with another molecule of alfa-ketoglutarate to form glutamate and alfa-ketoadipate. Alfa-ketoadipic acid undergoes oxidative decarboxylation to form glutaryl-CoA. The glutaryl-CoA is converted to crotonyl-CoA and to acetoacetyl-CoA.

The reactions are shown in Fig. 17-6.

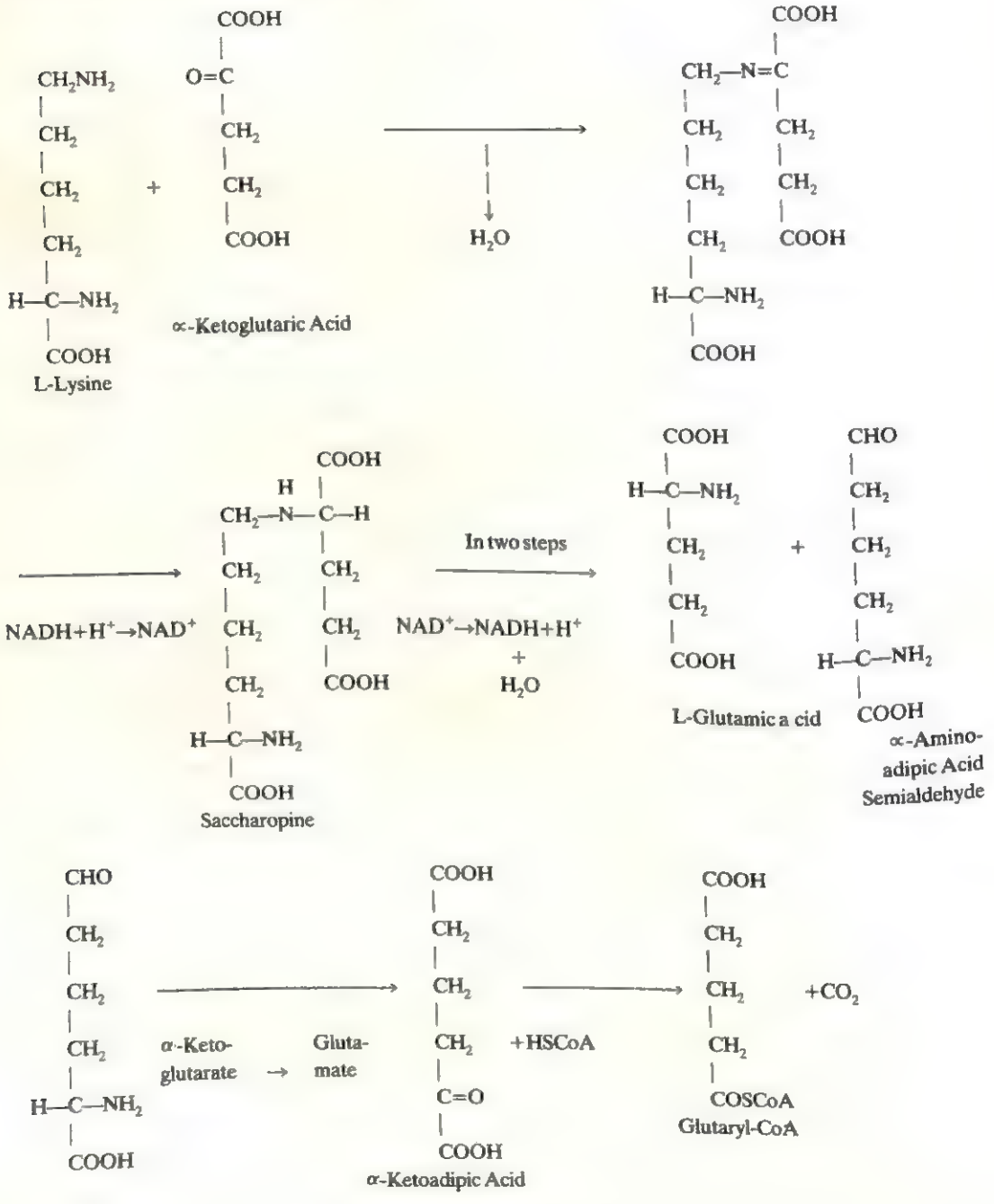


Fig. 17-6. Metabolism of lysine

Hydroxylysine is a constituent of collagen. The conversion of lysine to hydroxylysine occurs after lysine is incorporated into the collagen molecule.

Aspartic and Glutamic acids

The two belong to the acidic group (monoamino dicarboxylic acids). They are non-essential and glycogenic. Though they are non-essential because they can be readily synthesized in the body, they are the most active of all amino acids in metabolism.

They participate in transamination, transamidation and interconversion of amino acids. They also participate in ammonia transport and urea formation.

1. *Glycogenic function:* On deamination they form oxaloacetate and α-ketoglutarate, both of which are Kreb's cycle intermediates. Hence they can form glycogen.

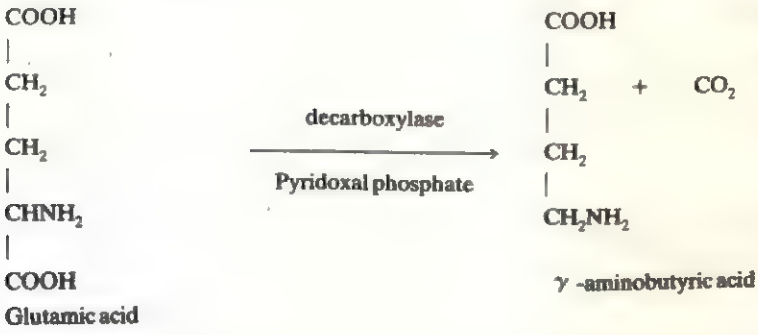
2. They are very active in transamination reactions as already seen under general metabolism of amino acids.

3. N-acetyl glutamate and aspartate are both active participants in urea formation (Krebs Henseleitt cycle).

4. They help in transport of ammonia by forming the corresponding amides aspargine and glutamine. This is of particular importance in brain.

5. In the CNS, glutamic acid also plays a key role in the transport of K^+ into the cells.

6. On decarboxylation, glutamic acid gives rise to gamma aminobutyric acid. This is a regulator of neuronal activity and depresses the activity. The formation of gamma aminobutyric acid and its subsequent oxidation require pyridoxal phosphate.



7. Glutamic acid is one of the constituents of glutathione which is necessary for activity of many of the sulfhydryl enzymes.

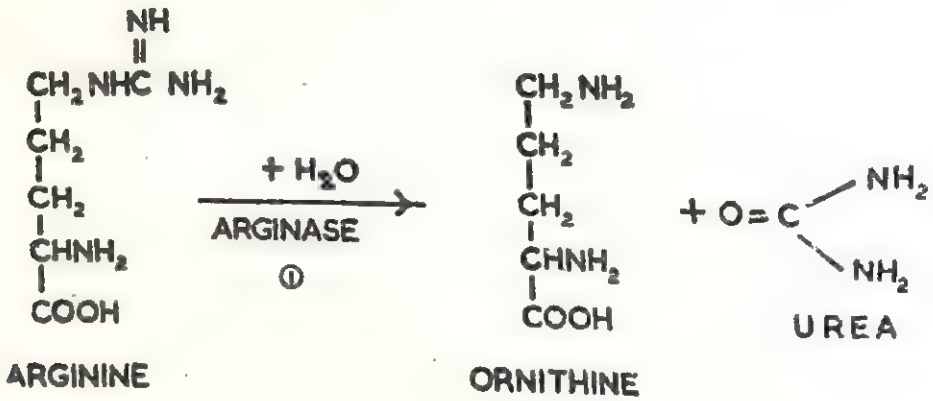
8. Aspartic acid participates in the synthesis of the purine and pyrimidine rings.

9. Glutamine is used in the higher animals for conjugation (detoxication with phenyl acetic acid).

10. Glutamate is a constituent of folic acid.

Arginine, Ornithine and Citrulline

Arginine is an essential amino acid. It can be dispensed with for short periods. The other two are non-essential. All three are basic. In addition to the amino group in the alfa position, arginine has a guanido group, ornithine an amino group and citrulline a ureido group attached to the delta carbon. All three are glycogenic. Ornithine and citrulline are formed during urea cycle while arginine is also a structural component of proteins.



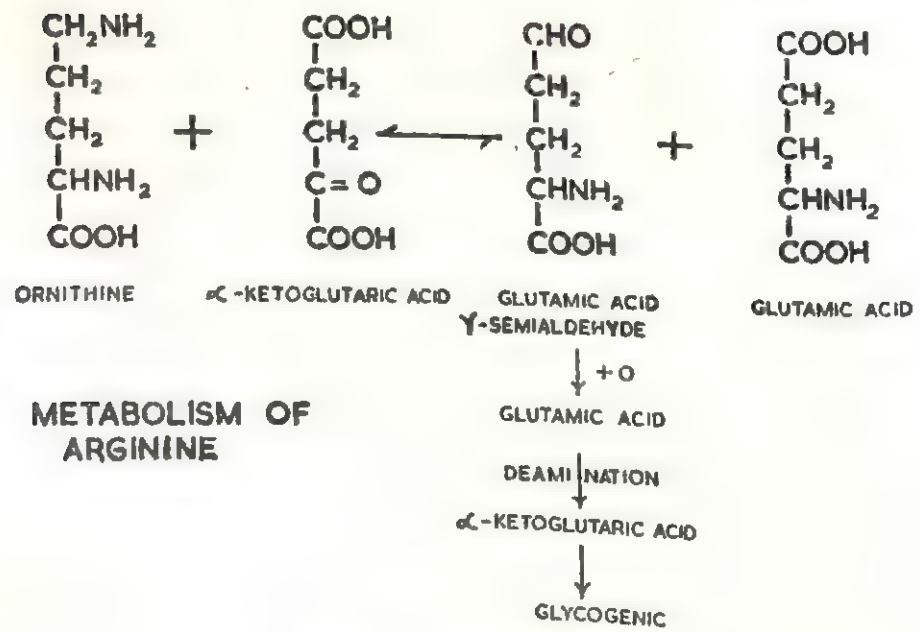


Fig. 17-7. Metabolism of arginine.

Functions of arginine:

1. The formamido group (—C—NH_2) can be transferred to glycine to form guanidoacetic acid (glycocyamine) which can be methylated to form creatine.

2. It is the immediate precursor in the formation of urea by the liver.

3. Arginine, by the action of arginase, is converted to ornithine and urea. The ornithine, on transamination, becomes converted to glutamic acid semialdehyde which can be oxidized to glutamate. Thus it is glycogenic. (For detailed reactions, see Fig. 17-7).

4. Ornithine, in conjunction with methionine, serves as a precursor for the synthesis of polyamines *spermidine* and *spermine*. These polyamines are growth factors and are required for cell proliferation. Since they carry a high positive charge, they readily associate themselves with polyanions like DNA and RNA and help in stabilizing those structures and may also stimulate their synthesis. They also act as inhibitors of certain enzyme synthases, particularly the kinases. In pharmacological doses, they act as hypothermics and sedatives.

Spermidine and spermine are oxidized to putrescine and other products by the enzyme *'polyamine oxidase'* which is present in the liver peroxisomes. Large amounts of putrescine and spermidine are excreted in urine in an acetylated form.

Proline and Hydroxyproline

Both are non-essential glycogenic amino acids. They can be readily formed from glutamic acid semialdehyde derived during the metabolism or ornithine. Hence they can be formed from ornithine, arginine and also from glutamic acid and all substances capable of forming glutamate (See Fig. 17-8).

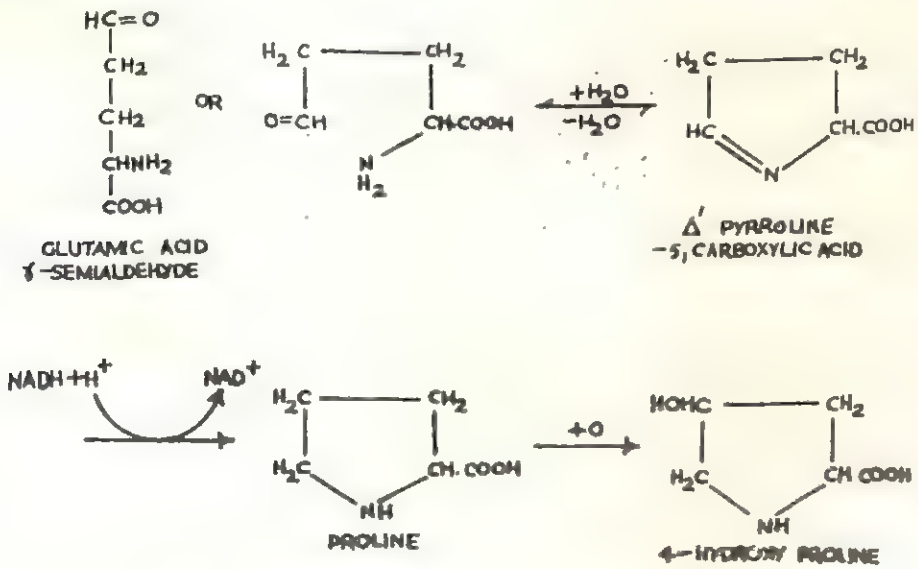


Fig. 17-8. Synthesis of proline and hydroxyproline.

The hydroxyproline is formed from proline after its incorporation into protein (as in the case of hydroxylysine). Proline is metabolized by a reversal of the synthetic reactions, forming glutamic acid and alfa ketoglutarate. Three of the 5 carbons of hydroxyproline are converted to pyruvate and the remaining two form glyoxylate. These reactions are not shown in the figure.

The amino acids are important constituents of collagen. Excessive amounts of hydroxyproline occur in blood and urine in a condition called Marfan's syndrome due to an inborn error in the metabolism of this amino acid.

Histidine

Like arginine, histidine also is relatively essential. An adult can withstand short term deprivation of the amino acid and still maintain a nitrogen balance. Long term deprivation in the adult and deprivation even for a short time in the growing individual are harmful. Its synthesis in the body is slow.

1. It is glycogenic through formation of glutamic acid. Ammonia and formate are the other products.

2. The formate can serve as a one carbon moiety.

3. In normal pregnancy there is some increase of histidine excretion in urine (histidinuria of pregnancy). Such an increase does not occur in toxemias of pregnancy. This is hence of diagnostic importance.

4. Decarboxylation of histidine produces histamine. The reaction is brought about by a specific histidine decarboxylase or a general aromatic L-amino acid decarboxylase. The latter enzyme produces tyramine from tyrosine, tryptamine from tryptophan and serotonin from 5-hydroxytryptophan. These are all toxic amines which cause a rise of blood pressure. Anti-hypertensive drugs produce an inhibition of this enzyme.

Histamine is converted to corresponding aldehyde and ammonia is liberated by the action of a diamine oxidase enzyme.

5. The R.B.C. and liver contain a histidine derivative called ergothionine. It is a reducing substance and gives a false high value for blood glucose by the ordinary methods of glucose estimation which employ reducing property of glucose.

6. Carnosine and anserine are two histidine derivatives present in muscle. Methyl derivatives of the amino acid occur in urine of patients suffering from myopathy (disease involving skeletal muscle). They are probably derived from carnosine and anserine.

7. Increased amounts of histidine in blood and urine and of anserine and carnosine and other incompletely metabolized products occur as inborn errors of metabolism in some.

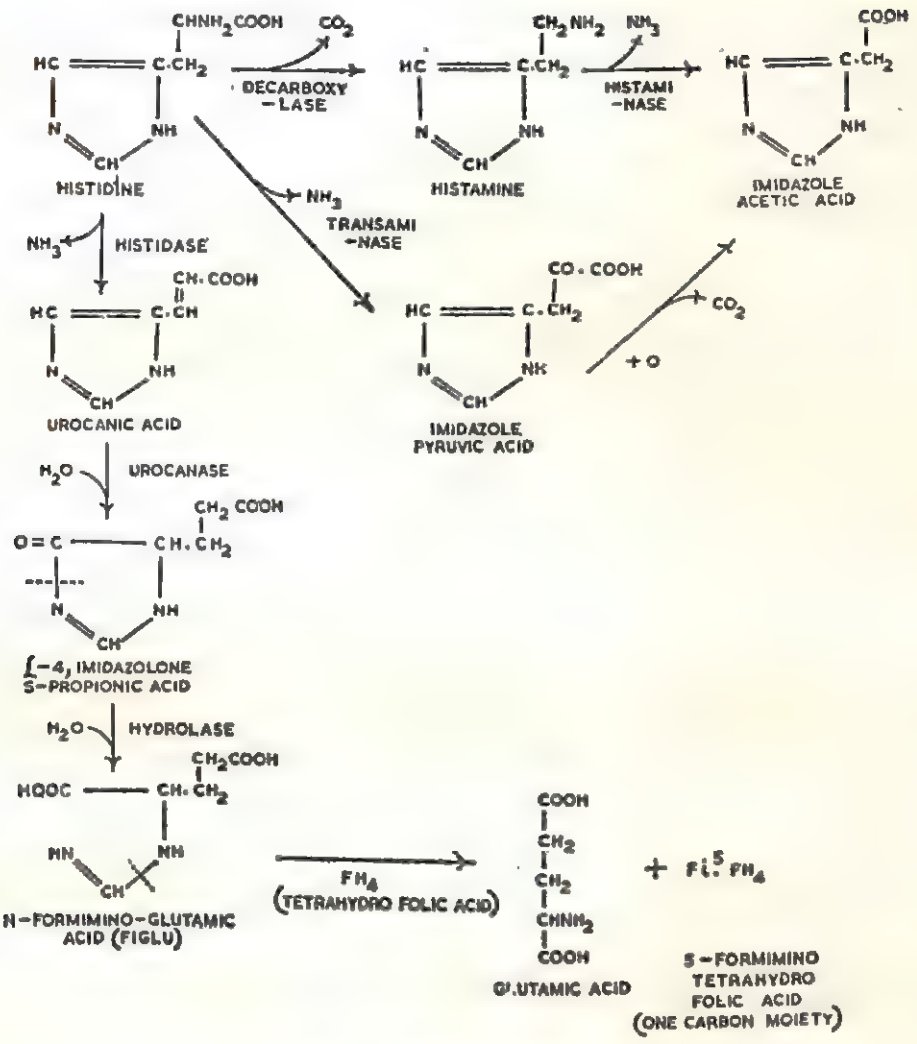


Fig. 17-9. Metabolism of histidine.

8. The one carbon fragment of histidine is taken up by folic acid and metabolized by transformylation reactions normally. In deficiency of folic acid, the histidine derivative, formiminoglutamic acid (figlu), is excreted in urine in large amounts and this is used as a test for folic acid deficiency.

The metabolic pathways of histidine are outlined in fig. 17-9.

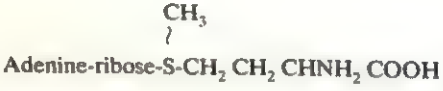
Methionine, Cysteine and Cystine

All three are sulfur containing amino acids, Methionine is essential while the other two can be readily synthesized. All are glycogenic. Methionine is particularly important as a donor of methyl group in reactions known as transmethylation reactions.

Active methionine: To act as methyl donor, the amino acid has to be first activated by ATP.

$$\text{Methionine} + \text{ATP} \xrightarrow[\text{(liver)}]{\text{activating enzyme}} \text{active methionine} + \text{PP} + \text{P}$$

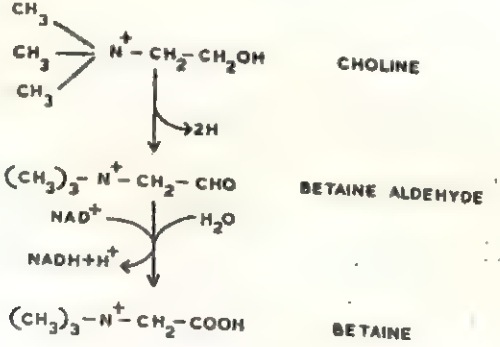
Active methionine or adenosyl-S-methionine has the following structure:



The S~methyl bond is a high energy bond. The methyl group is hence labile and can be readily transferred to an acceptor (Fig. 17-10.I). The activiating enzyme is known as *methionine-adenosyl transferase*'.

The enzymes which bring about transmethylation are called *methyl transferases* or *methylferases.*

Betaine is another important donor of methyl groups. It is formed from choline as shown below.



Some examples of transmethylation reactions

Methyl donors	*Methyl acceptors*
S-adenosyl methionine and betaine ↓ ~CH_3 (labile methyl group)	1. Ethanolamine +CH_3 ↓ Methylethanolamine +CH_3 ↓ Dimethylethanolamine +CH_3 ↓ Trimethylethanolamine (Choline)
	2. Guanidoacetate+CH_3→Creatine
	3. Pyridine+CH_3→N-Methylpyridine
	4. Norepinephrine+CH_3→Epinephrine
	5. Homocysteine+CH_3→Methionine
	6. Cytosine+CH_3→5-Methylcytosine
	7. Uracil+CH_3→Thymine

By a transulfuration reaction requiring pyridoxal phosphate as a coenzyme, the homocysteine, formed from methionine, interacts with serine to form homoserine and cysteine. A compound, cystathionine, (a complex of serine and homocysteine), is formed as an intermediate. The homoserine can be metabolized to form alfaketobutyrate which is then converted to propionyl-CoA, methylmalonyl-CoA and finally succinyl-CoA.

Cysteine is metabolized as shown in fig. 17-10, II & III.

The main pathway is towards formation of pyruvate by desulfhydration and deamination.

A subsidiary path of oxidation of the –SH to sulfate followed by decarboxylation will yield taurine. Taurine is used in conjugation of cholic acid to form one of the bile acids — taurocholic acid.

Functions of Cysteine:

1. The readily reversible oxidation-reduction reaction cystine⟷cysteine helps keep the sulfhydryl enzymes in the reduced and active state.

2. Cysteine is used for conjugation (detoxication) with halogenated aromatic compounds (e.g.: bromobenzene).

3. It is a constituent of glutathione which is glutamyl-cystinyl-glycine. The active group of this tripeptide is the –SH group. It is hence simply represented as GSH and like cysteine, it can also exist as GS-SG, the oxidized form and GSH the reduced form. It keeps the enzymes in the active –SH form.

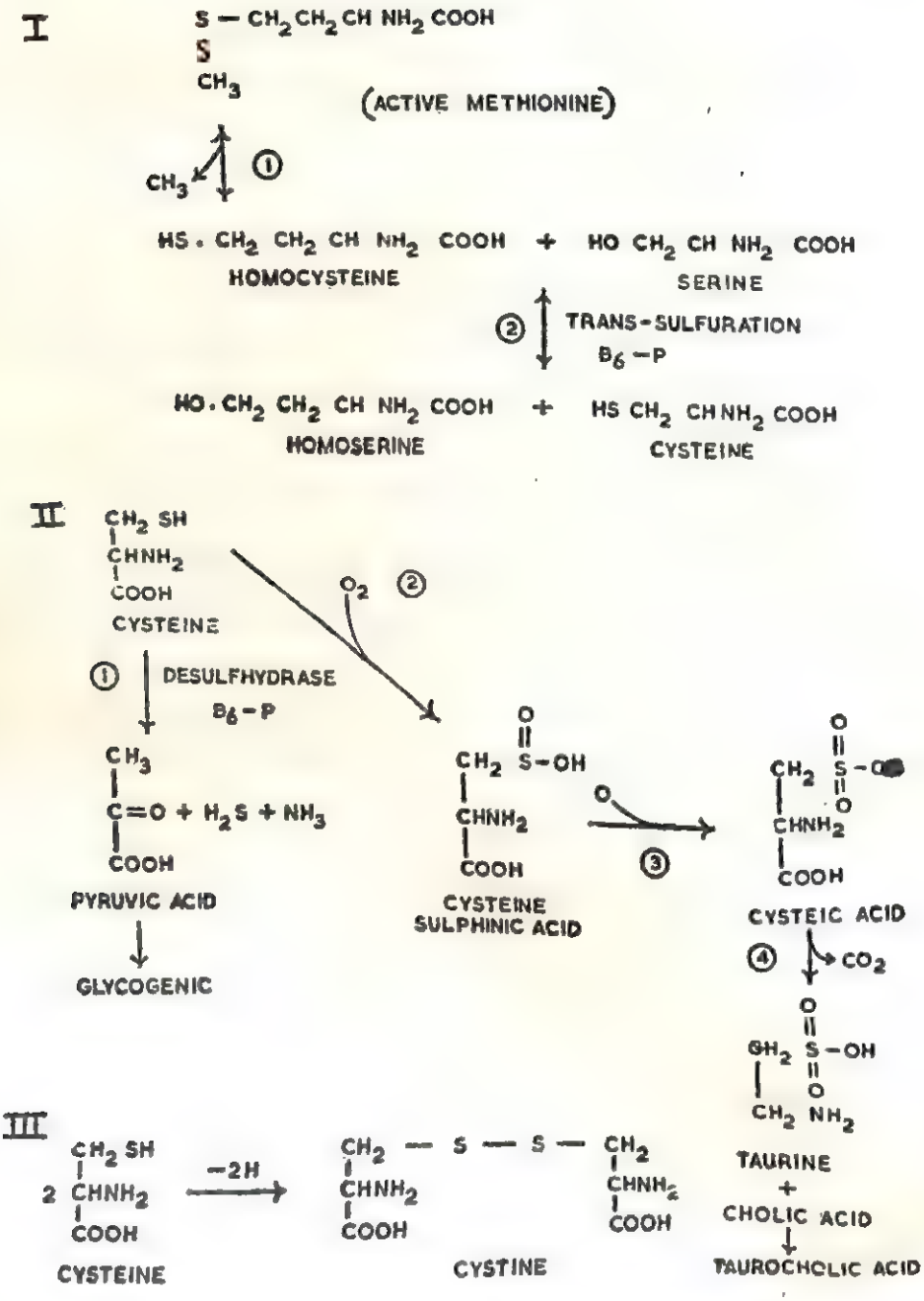


Fig. 17-10. Metabolism of sulfur containing amino acids.

4. Taurine produced from cysteine is a constituent of the bile acid taurocholic acid.

5. Cysteine is an important constituent of scleroproteins e.g.: keratin of skin, hair and nails.

6. -S-S-linkages between neighbouring cysteine molecules in polypeptide chains are partly responsible for the secondary and tertiary structures of protein molecule.

Abnormalities in metabolism of sulfur containing amino acids:

Cystinuria: Large amounts of diamino acids — lysine, arginine, ornithine and cystine — are excreted in urine. Cystine being the most insolube of all, it is readily precipitated and the crystals can be seen under microscope. Hence it is called cystinuria. Calculi containing cystine may be formed in the tubules and elsewhere in the urinary passages. There seems to be an inability in the reabsorption of these diamino acids by the renal tubule.

Cystinosis: Cystine is deposited in the reticuloendothelial system. There is an increased excretion of all amino acids in urine.

Homocystinuria: The homocysteine formed from methionine cannot be converted to cysteine with the result it accumulates in tissues and blood and is excreted in urine.

Normal excretion of sulfur in urine: Normal urine contains sulfur in three forms.

1. **Inorganic sulfate:** The sulfur is completely oxidized to sulfate and excreted as sulfate. This accounts for 80% of the urinary sulfate.

2. **Ethereal sulfate:** The sulfate conjugates with phenolic substances, indole and skatole, mostly formed in the intestine during putrefaction of amino acids, and is excreted.

This is a detoxication mechanism and accounts for 5% of urinary sulfate.

3. **Organic sulfur.** This forms 15% of the total. It consists of sulfur containing amino acids and mercaptans.

Phenylalanine and Tyrosine

These two are aromatic acids containing a benzene ring. While phenylalanine is an essential amino acid and cannot be synthesized in the body, tyrosine can be readily formed from phenylalanine and is hence non-essential. In fact the normal metabolic pathway of phenylalanine is through conversion to tyrosine.

1. The amino acids are both glycogenic and ketogenic (See fig. 17-11)

2. The hormones, noradrenaline and adrenaline, are synthesized from 3, 4-dihydroxyphenylalanine (DOPA), a product formed by oxidation of these amino acids. (See fig. 17-12).

3. The pigment, melanin, which is present in the skin, hair and iris is also formed from DOPA.

4. Throxine is formed by iodination and coupling of two molecules of iodinated tyrosine.

Most of phenylalanine and tyrosine is oxidized to homogentsic acid which, in the liver, is further converted to fumaric acid and acetoacetic acid by opening of the benzene ring.

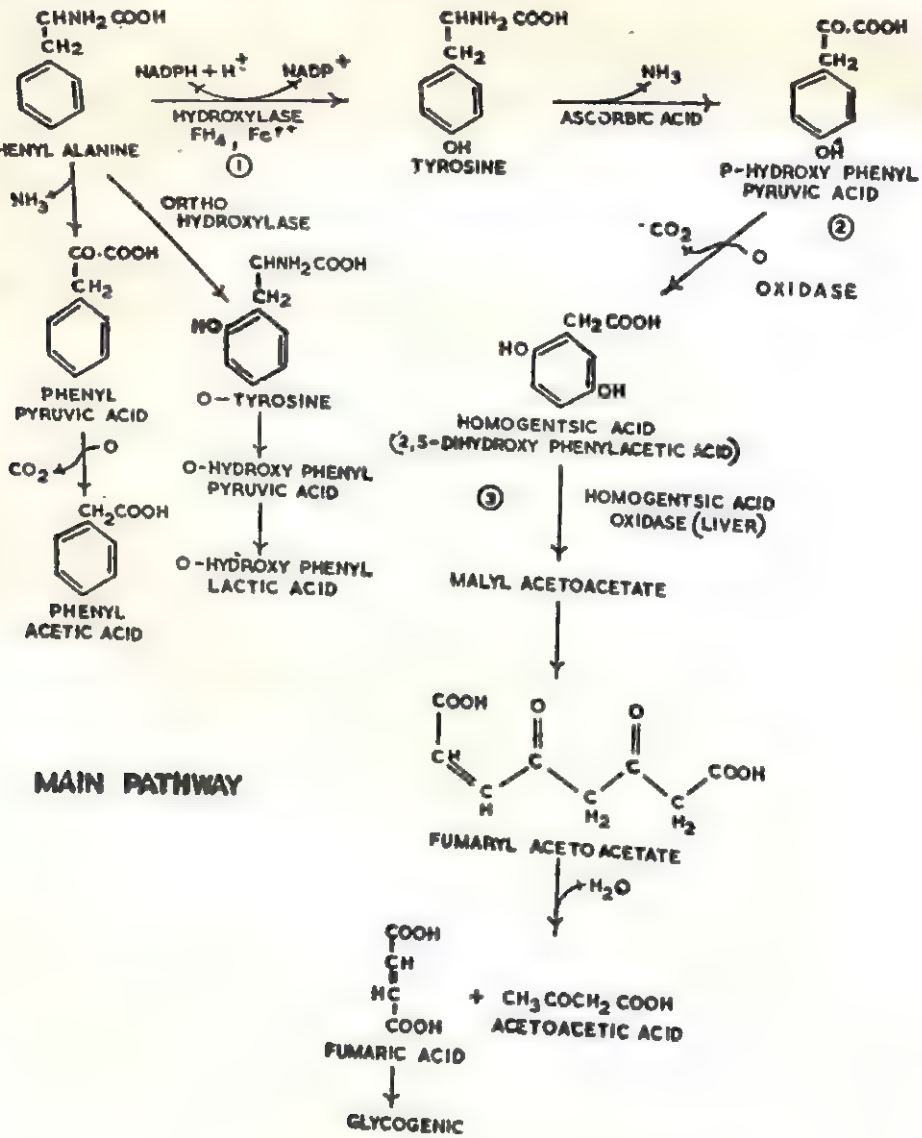


Fig. 17-11. Metabolism of phenylalanine and tyrosine (main pathway).

Fumaric acid is oxidized via citric acid cycle and is glycogenic. The acetoacetate, a ketone body, leaves the liver and is utilized in the tissues. Conversion to tyramine by decarboxylation occurs mainly in the intestines during putrefaction. The enzymic conversion of parahydroxyphenylpyruvate to homogentsic acid requires ascorbic acid and copper ions.

Homogentsic acid oxidase requires iron as activator. Several abnormalities occur in the above metabolic pathways.

1. Phenylketonuria: The hydroxylation of phenylalanine in the para position to form tyrosine is mediated by the enzyme phenylalanine hydroxylase, requiring Fe^{++} and $NADPH+H^+$ as coenzyme.

Tetrahydrobiopterin, a compound similar to tetrahydrofolate is also required for the reaction. Tetrahydrobiopterin loses two hydrogen atoms in the reaction and is converted to dihydrobiopterin. It is reconverted to tetrahydro form by accepting hydrogens from $NADPH+H^+$.

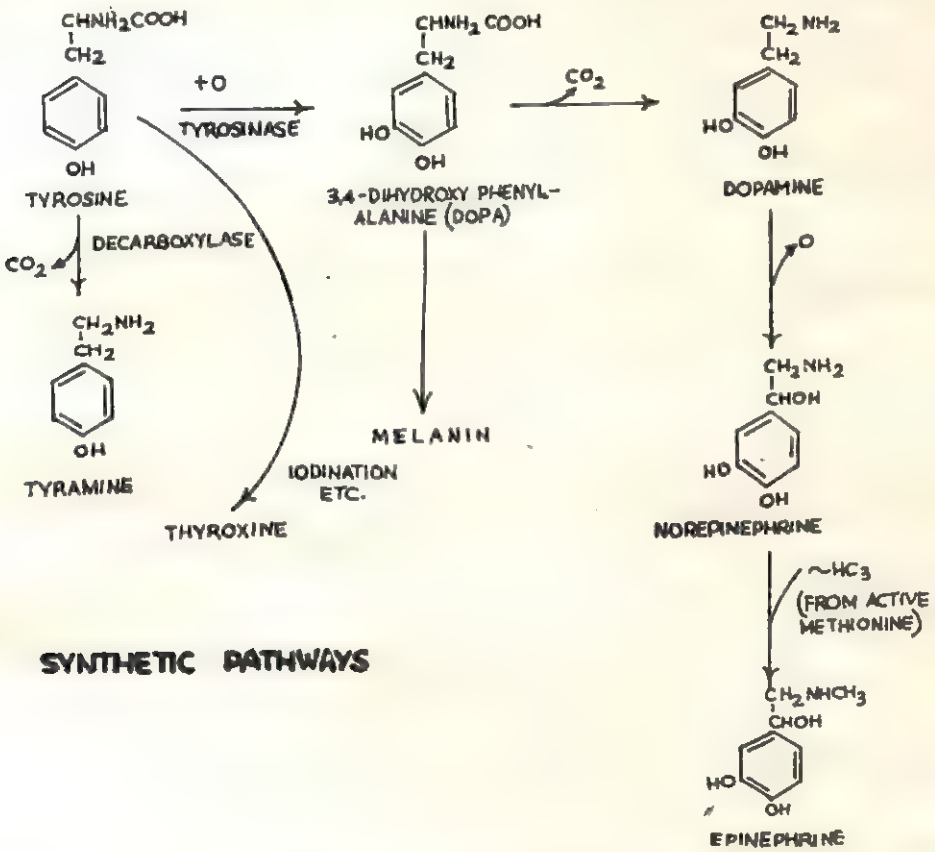


Fig. 17-12. Metabolism of phenylalanine and tyrosine (synthetic pathways).

The enzyme is present in liver. In phenylketonuria the enzyme is absent. Hence phenylalanine is converted to phenylpyruvic, lactic and acetic acids which cannot be metabolized further and are excreted. Also an abnormal orthohydroxy derivative is formed whose metabolites also may be found in the urine. The condition occurs as an inborn error of metabolism in children and is associated with mental symptoms. There also appears to be an associated deficiency in hydroxylation of tryptophan to form serotonin.

2. Tyrosinosis: In the formation of homogentsic acid from tyrosine, there is a shift in the position of -OH from 3rd to 2nd position and introduction of a new -OH at 5th position along with oxidation of side chain. Inability to form homogentsic acid due to lack of the enzyme para-hydroxyphenylpyruvic acid oxidase leads to accumulation of tyrosine in tissues and blood and its excretion in urine.

3. Alkaptonuria: Here the block is in the opening of the ring to form mallyl-acetoacetate. The enzyme, homogentsic acid oxidase, is absent in the liver. Hence homogentsic acid (alkapton) is excreted in urine. On standing, the urine darkens due to further oxidation of homogentsic acid.

In long standing cases, deposition of homogentsic acid in the cartilages of ear and other exposed places may darken them — a condition called 'ochronosis'.

Alkaptonuria may also occur in premature infants on account of vitamin C deficiency and can be cured by administering the vitamin.

The benzene ring of phenylalanine and tyrosine contributes to phenolic sulfates in the urine classed as ethereal sulfates.

4. Albinism: This is a condition where there is no pigment in the skin, hair and iris leading to an unhealthy white color of these structures. It is due to inability to convert DOPA to melanin due to absence of tyrosinase in the melanocyte cells that are concerned with the production of the pigment.

Tryptophan

Tryptophan is an essential amino acid. It is both glycogenic and ketogenic. Its keto acid can be aminated and hence replace the amino acid in diet.

1. The main pathway of its metabolism is by way of anthranilic acid which can be finally converted to glutaric acid which in turn gives two molecules of acetate.

2. Small amounts of the amino acid can also be converted to nicotinic acid in the body. From every 60 mg. of tryptophan about 1 mg. of niacin is obtained. To that extent, tryptophan-rich diets have a sparing effect on niacin requirements.

3. Decarboxylation of the side chain and hydroxylation at position 5 of the indole nucleus forms 'serotonin' or 5-hydroxytryptamine which is a potent vaso-constrictor, smooth muscle constrictor and stimulator of cerebral activity. It is formed in the intestinal epithelium, blood platelets and in the brain. In tumours known as argentaffinomas involving the serotonin forming cells of intestinal epithelium, large amounts of this material are formed and excreted after oxidation of side chain to form 5-hydroxyindole acetic acid (5 HIAA). Normal adults excrete about 7 mg. of HIAA per day. In malignant carcinoid, the excretion may increase to as much as 400 mg. a day.

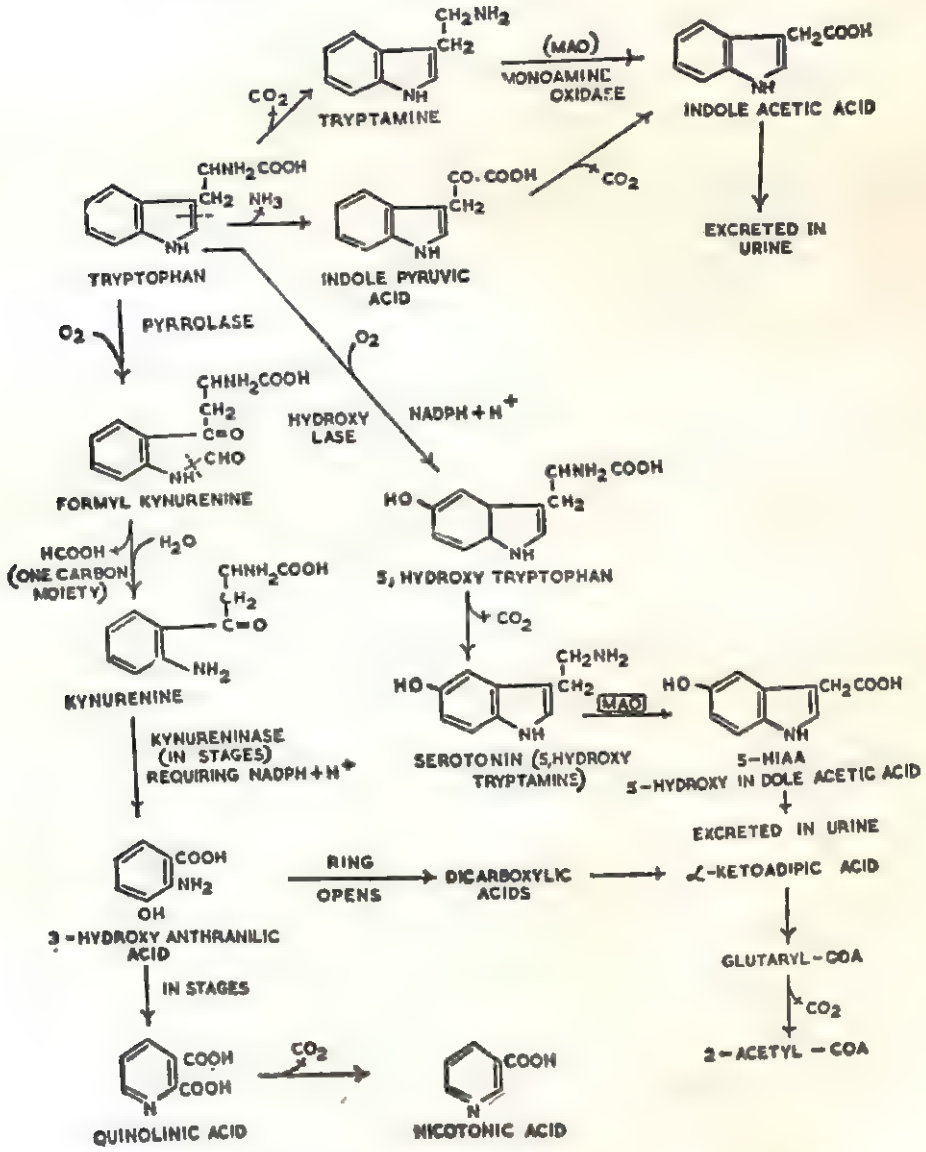


Fig. 17-13. Tryptophan metabolism

The enzyme which catalyzes the conversion of serotonin to 5-HIAA is called monoamine oxidase (MAO). Drugs which inhibit this enzyme (*e.g.* iproniazid — isopropyl isonicotinyl hydrazine) will prolong serotonin action on the brain and produce a psychic stimulation due to increased cerebral activity.

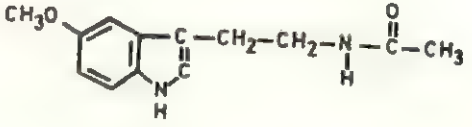
The serotonin of the brain is in a bound form. Reserpine, the antihypertensive drug, acts by releasing the serotonin from its bound form and thus making it readily available to the monoamine oxidase action. Hence reserpine produces a depression of cerebral activity.

The metabolic pathways of tryptophan are schematically presented in Fig. 17-13.

The tryptophan pyrrolase required in the oxidation of tryptophan to formyl-kynurenin is an enzyme similar to the phenylalanine hydroxylase. It is a dioxygenase. It requires copper and ferriheme for its activity. A deficiency of vitamin B_6 will interfere with normal functioning of kynureninase and leads to the production of xanthurenic acid from kynurenin. Urinary indican is derived from indole and skatole absorbed from intestine due to putrefaction involving the amino acid tryptophan.

Hartnup disease: The disease resembles pellagra (niacin deficiency) and manifests skin lesions, cerebellar ataxia and mental changes similar to pellagra. This is associated with a defect in the renal and intestinal transport of tryptophan. Large amounts of the amino acid are excreted in the urine and feces. Indole derivatives are also increasingly excreted. There is also a block in the conversion of tryptophan to kynurenin and nicotinic acid.

Melatonin: Melatonin, the hormone of the pineal body and the peripheral nerves of man, is synthesized from serotonin. It is N-acetyl, 5-methoxy-serotonin.



MELATONIN

CREATINE AND CREATININE METABOLISM

Creatine occurs in the free form and as creatine-phosphate in muscle, brain, liver, kidneys and blood. It is converted to creatinine in muscle and excreted in urine.

Striated muscle contains about 1 g. creatine per 100 g. Half of this is in the form of creatine phosphate.

The steps in the formation of creatine and creatinine are shown in Fig. 17-14.

Normal adult urine contains creatinine in amounts directly proportionate to body weight (mainly on account of muscle weight). A 70 kg. man excretes about 1.5 grams per day. Women excrete less (being less muscular). Creatine is present only in traces in adult urine. Premature infants and infants during the first few days of life excrete large amounts of creatine in urine. It is also excreted in large amounts in diseases involving skeletal muscle (myopathies), hyperthyroidism, diabetes mellitus and other wasting diseases.

Women excrete small amounts of creatine during menstrual period due to its release from the smooth muscle cells of the endometrium.

Normal blood plasma contains 0.2–0.6 mg. of creatine and 0.5–1.0 mg. of creatinine per 100 ml.

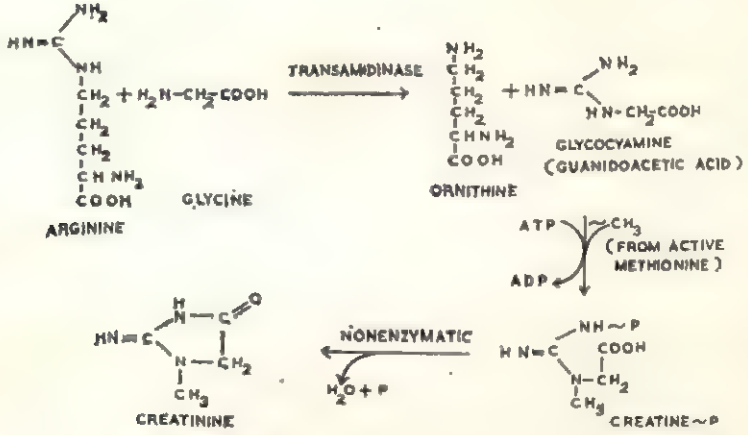


Fig. 17-14. Formation of creatine and creatinine.

References:

Anfinsen, C.B.: The Molecular Basis of Evolution, Wiley, N.Y., 1959.
Baldwin, E: Dynamic Aspects of Biochemistry, 3rd ed., Cambridge University Pr., N.Y.. 1957.
Campbell, P.N. and Sargent, J.R. (eds): Techniques in Protein Biosynthesis; Acad. Pr., N.Y., 1966.
Frish L. (ed): The Genetic Code; Cold Spring Harbour Symp. Quant. Biol. 31: 1966.

Gross, F. (ed): Protein Metabolism; Springer-Verlag OHG, Berlin, 1962.
Hsai, D.Y.: Inborn Errors of Metabolism, Yearbook Medl., Chicago, 1966.
Ingram, V.M.: The Biosynthesis of Macromolecules. Benzamin, N.Y., 1965.
Meister, A.: Biochemistry of the Amino Acids, 2nd Ed., Vols. 1 and 2, Acad. Pr., N.Y., 1965.
Perutz, M.F.: Proteins and Nucleic Acids; American Elsevier, N.Y., 1962.
Schonheimer, R.: The Dynamic State of Body Constituents, Harvard University Pr., Cambridge (Mass), 1942.
Stanbury, J.B., Wyngaarden, J.B., and Fredrickson, D.S. (eds).: Metabolic Basis of Inherited Disease, 2nd Ed., McGraw-Hill, N.Y.: 1966.
Watson, J.D.: Molecular Biology of the Gene, Benzamin, N.Y., 1965.
Wiseman, A.: Organization for Protein Biosynthesis, American Elsevier, N.Y., 1965.

18

INTEGRATION OF CARBOHYDRATE, LIPID AND PROTEIN METABOLISMS

THOUGH each of these metabolisms had been considered separately for the sake of convenience, they take place simultaneously in the intact organism and are closely interrelated to each other. The metabolic processes involving these three types of materials can be broadly divided into three stages.

1st Stage: *Stage of hydrolysis to simple units:*

The polysaccharide, glycogen, is broken down to glucose. The lipids are hydrolyzed to glycerol and fatty acids. The proteins are hydrolyzed to amino acids. This is the prelude to either further synthesis of new substances or for their oxidation. Very little of energy is produced in this hydrolytic phase and it is dissipated away as heat. There is no storage of energy.

2nd Stage: *Preparatory stage:*

The monosaccharide, glucose, runs through glycolytic reactions to produce the three carbon compound, pyruvic acid, which, in turn is converted to acetyl-coenzyme A. The glycerol of triglycerides is also converted, by entering the same glycolytic pathway, to pyruvate and then acetyl-Coenzyme A.

The fatty acids undergo β-oxidation and form several molecules of acetyl-coenzyme A.

The amino acids are deaminated or transaminated first and the carbon skeleton is metabolized differently from amino acid to amino acid. In the case of alanine, serine and cysteine, pyruvic acid is formed as in case of carbohydrates and glycerol. This is then converted to acetyl-coenzyme A. In the case of glutamic acid, proline, histidine etc., alfa-ketoglutarate is formed through which they enter the citric acid cycle direct. Yet a few others like leucine, phenylalnine, and tyrosine yield acetate or acetoacetate which can be converted to acetate.

During the second phase (glycolysis, β-oxidation etc.) also relatively small amounts of energy are produced and this is stored as ATP.

3rd Stage: *Oxidative stage, citric acid cycle or the aerobic final pathway of metabolism:*

The carbohydrates, lipids and proteins – all form acetate or some other intermediate like oxaloacetate, alfa-ketoglutarate, succinate or fumarate of the citric acid cycle. Having gained

entry into the cycle at any site, 2 of the carbons of citrate constituting an acetate moiety are oxidized finally to carbondioxide and water and the energy of oxidation by the electron transport chain is captured as energy-rich phosphate–ATP mostly. This yields the largest amount of energy of all the three stages.

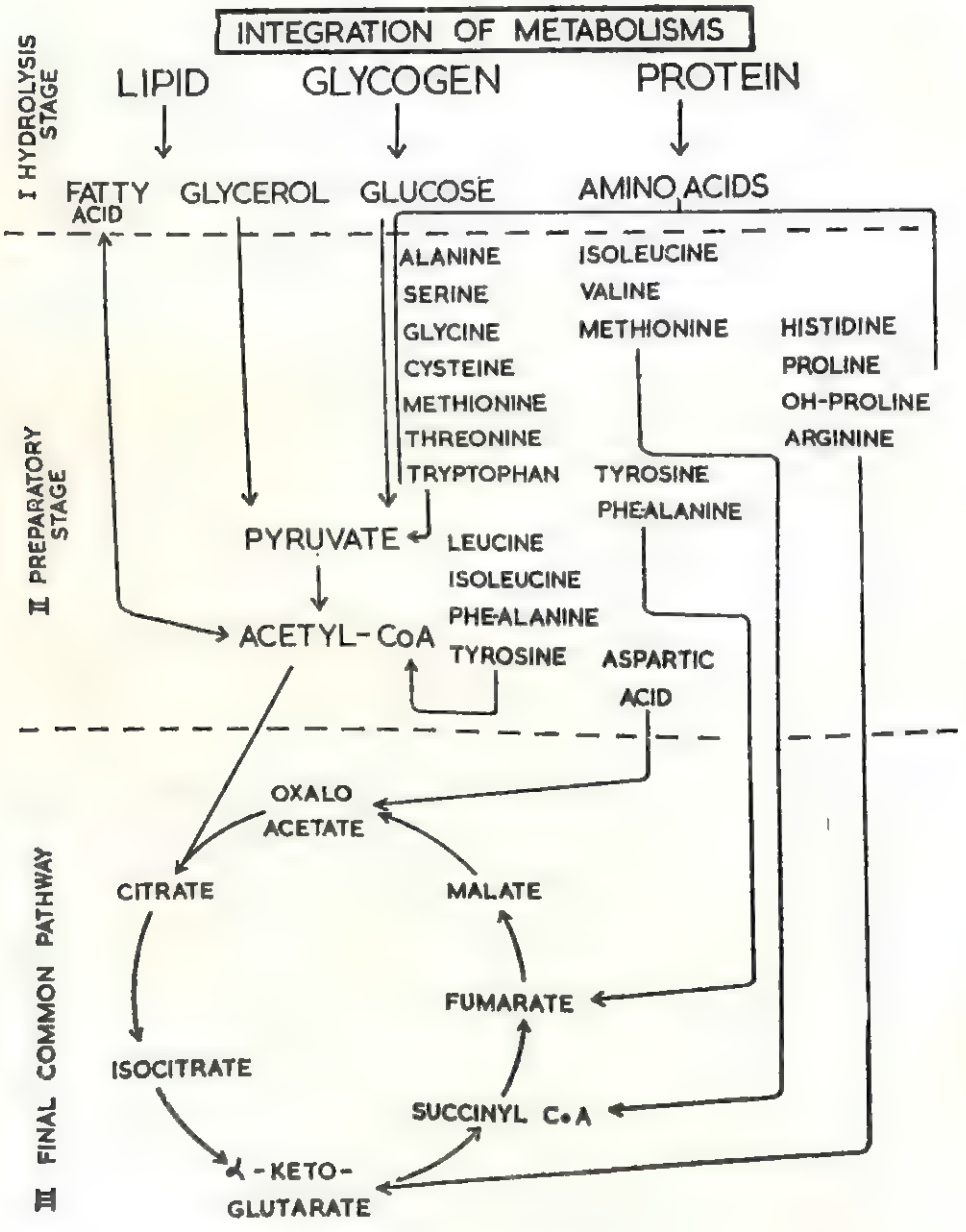


Fig. 18-1. Integration of metabolisms.

Thus the pathways are similar to a large extent and identical in the final stage of oxidation of the metabolites, whether derived from carbohydrate, lipid or protein. This is schematically represented in fig. 18-1.

INTERCONVERSION BETWEEN THE THREE PRINCIPAL COMPONENTS

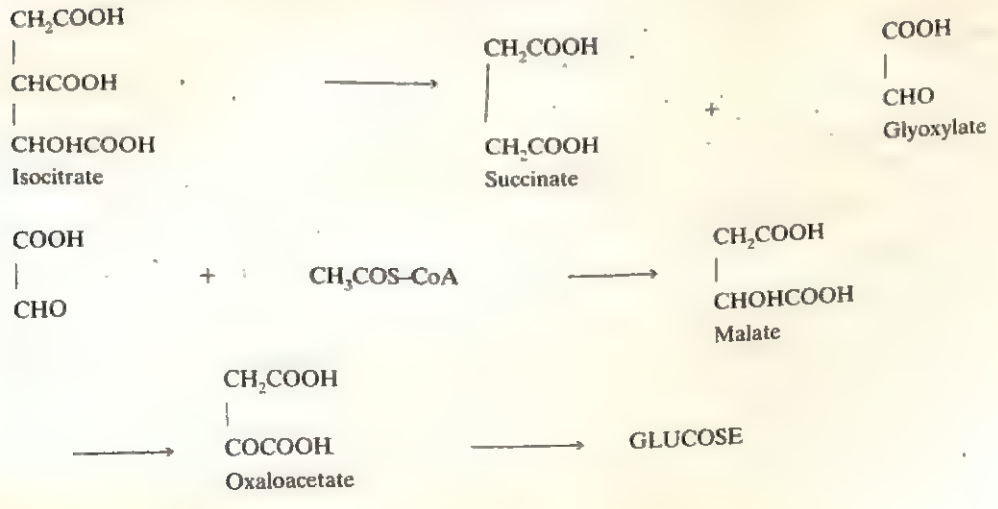
I. (i) Carbohydrates can form lipids through formation of
(a) Glycerol from dihydroxyacetone phosphate (glycolysis) and
(b) Fatty acid from acetyl-CoA.

(ii) Carbohydrates can form the non-essential amino acids through amination of the α-ketoacids like pyruvic, oxaloacetic and α-ketoglutaric acid to form alanine, aspartic acid and glutamic acid.

II. (i) Fatty acids can be converted to some amino acids by forming the dicarboxylic acids like malic, oxaloacetic and α ketoglutaric acids.

(ii) Fatty acid carbon may theoretically be incorporated into carbohydrate by the acetate running through citric acid cycle. But there is no net gain in carbohydrate since two carbons–equivalent of acetate–are oxidized in the cycle. However acetate can form glucose by running through the glyoxylate cycle. The glyoxylate cycle does not occur in the mammalian tissues. Hence, acetate does not cause a net increase in glucose content in animals, though the acetate carbons do get incorporated into the glucose molecule.

In micro-organisms and in plant seedlings, isocitrate, instead of conversion to alfaketoglutarate in the citric acid cycle, is broken down directly to succinate and glyoxylate. The glyoxylate can condense with acetyl-CoA to form malate which in turn is converted to oxaloacetate. This can now give rise to glucose through gluconeogenesis. The reactions are as follows:



III. Protein can form both carbohydrate and lipid through its glycogenic and ketogenic amino acids.

REGULATION AND CONTROL OF THE REACTIONS

Regulation of metabolic processes can occur at the level of 1. the cell 2. an organ or 3. the whole organism. The nervous and circulatory systems help as channels of communication between cell to cell and organ to organ in the higher organisms. The hormones have an extensive role to play in these regulatory mechanisms.

General Regulation of Metabolim:

As already seen, in most metabolic pathways, there are one or more irreversible reactions which help to drive the metabolic reactions towards the chosen goal; eg., hexokinase, phosphofructokinase and pyruvate kinase in glycolysis.

In other cases, where there is need to have two alternate metabolites depending on the prevailing conditions, the enzyme reaction or sequence of reactions are freely reversible, eg., lactic dehydrogenase can form pyruvate or lactate depending on whether the conditions are aerobic or anerobic.

Specific Mechanisms of Regulation:

1. *Entry of metabolites into the cell:* The cell membrane allows certain substances only to enter the cell (selective permeability), eg. erythrocyte membrane. There is also active transport of certain substances (eg. amino acids, glucose etc.). The transport involves expenditure of energy by the cell. The active transport may be altered or controlled by hormones (eg., glucose transport by insulin).

2. *Induction and repression of enzyme synthesis:* An added substrate may rapidly induce the synthesis of the enzyme concerned with its metabolism. This is particularly seen in bacteria. Conversely, the end product of a metabolic sequence may repress the enzymes required for its own synthesis. Addition of, say, abundant amounts of leucine to a culture of microorganisms will repress in those organisms the enzymes concerned with leucine synthesis.

3. *Feed-back inhibition:* This can limit the synthesis of metabolites only to the extent required. CTP inhibiting aspartate transcarbamylase and plasma cholesterol levels inhibiting HMG-CoA reductase levels are examples.

4. *Stimulation of enzymes by metabolites can also occur:*

While these processes can regulate the rate and direction of metabolic reactions, in individual cell or viscera, overall direction and information are derived through hormones. They operate on the target cell by one or the other of the mechanisms described above. The hormone, on reaching the target cell, seems to act by changing the local concentration of a substance called the *cyclic AMP*–adenosine-3', 5'-cyclic monophosphate. The cyclic AMP is formed from ATP by the enzyme adenyl cyclase (also called cyclic AMP synthetase), an

enzyme located in the plasma membrane. While the hormone is considered as the *first-messenger*, cyclic AMP may be cosidered as the *second messenger*. The cyclic AMP is inactivated by conversion to 5' AMP by an enzyme cyclic nucleotide phosphodiesterase. Several enzymes are sensitive to changes in the concentration of cyclic AMP in the cell. Cyclic AMP can thus play a major role in the regulation of the cellular metabolism. Its effects on glycogen synthetase and phosphorylase enzymes was already considered in detail in carbohydrate metabolism.

The ratio of ATP/AMP of the cell or tissue seems to decide the extent of its aerobic metabolism. If the ratio is high (low AMP level), the activity of isocitric acid dehydrogenase seems to be lowered resulting in an accumulation of citrate. The oxidation in the citric acid cycle decreases and ATP production falls. Also the increased citric acid levels stimulate acetyl-CoA carboxylase activity to convert acetyl-CoA to malonyl-CoA, the first step in fatty acid synthesis. Thus the acetyl-CoA, in the presence of adequate stores of ATP and low AMP levels, is diverted to the synthesis of fat. The reverse set of conditions operate where the ATP/AMP ratio is low.

A high level of ATP and low level of AMP also inhibit phosphofructokinase and thereby inhibit glycolysis. There results an accumulation of the hexosephosphate which interacts with UTP to form UDP-glucose and proceeds on to glycogen synthesis. The converse happens with low ATP and high AMP levels.

Reference:

Krebs, H.A. Kornberg. H.L.: Energy Transformations in Living Matter, Survey, Springer-Verlag, Berlin, 1957.

19

PORPHYRIN METABOLISM

THE porphyrins are complex structures consisting of four pyrrole rings united through methylene bridges. The nitrogen of the pyrrole rings can complex with metallic ions like iron and magnesium. They form the prosthetic groups of conjugated proteins–hemoglobin of the mammalian erythrocytes, myoglobin of the muscle, erythrocrurins of the invertebrates, cytochromes, and the enzymes catalase and peroxidase and other oxidative enzymes like tryptophan pyrrolase. All contain iron-poryhyrins as the prosthetic groups. Chlorophyll contains magensium-porphyrin as the prosthetic group.

Synthesis of porphyrins: The synthesis starts by a condensation between succinyl-CoA (active succinate) and glycine to form alfa-amino, β-ketoadipic acid, which is then decarboxylated to form δ-aminolevulinic acid. The enzyme aminolevulinic acid synthetase (ALA synthetase) requires pyridoxal phosphate as coenzyme and the reaction occurs in the mitochondria. Pantothenic acid is also required as a consituent of coenzyme-A. (See fig. 19-1). Mg^{++} acts as activator.

ALA synthetase is the rate limiting enzyme in heme synthesis. Decreased amounts of heme favour increased synthesis of the enzyme protein and vice versa.

Many of the insecticides and carcinogens, when they enter the human body, are metabolized in the liver using cytochrome P-450. Heme is therefore diverted for the synthesis of cytochrome P-450. To provide the heme required for regular hemoglobin production, ALA synthetase production is stimulated. Iron and steroid hormones also stimulate the enzyme production.

Two molecules of the δ-aminolevulinic acid combine to form a molecule of porphobilinogen, the precursor of the pyrrole ring. This step occurs in the cytoplasm and is mediated by the enzyme 'δ-aminolevulinase' (ALA dehydrase).

ALA dehydrase requires Zn^{++} as activator and is inhibited by lead.

Four such porphobilinogen molecules combine by methylene bridges derived from the alfa carbon of one of the two glycine molecules with elimination of one of the two amino groups as ammonia. The other alfa carbon is incorporated as one of the alfa carbons of the porphobilinogen and the amino nitrogen as the pyrrole nitrogen. The enzyme which brings about this tetrapyrrole condensation is porphobilinogen deaminase. The first porphyrin compound formed has therefore alternating acetate and propionic acid from positions 1 to 8. This is known as type I uroporphyrinogen. If C and D rings are combined in such a way as to give APPA in positions 5, 6, 7 and 8, Type III uroporphyrinogen is formed.

The subsequent steps take place in the mitochondria. Decarboxylation of the 4 acetates to form methyl groups will give rise to corresponding coproporphyrinogens. Removal of one atom of hydrogen from each methylene bridge and from two of the 'NH' groups (total 6H) will produce corresponding porphyrins. The type III coproporphyrin then undergoes further change to decarboxylate and further oxidize the propionic side chains in positions 2 and 4 to form vinyl groups.

$$\underset{\text{Propionate}}{-CH_2CH_2COOH} \longrightarrow \underset{\text{Vinyl}}{-CH{=}CH_2} + CO_2 + 2H$$

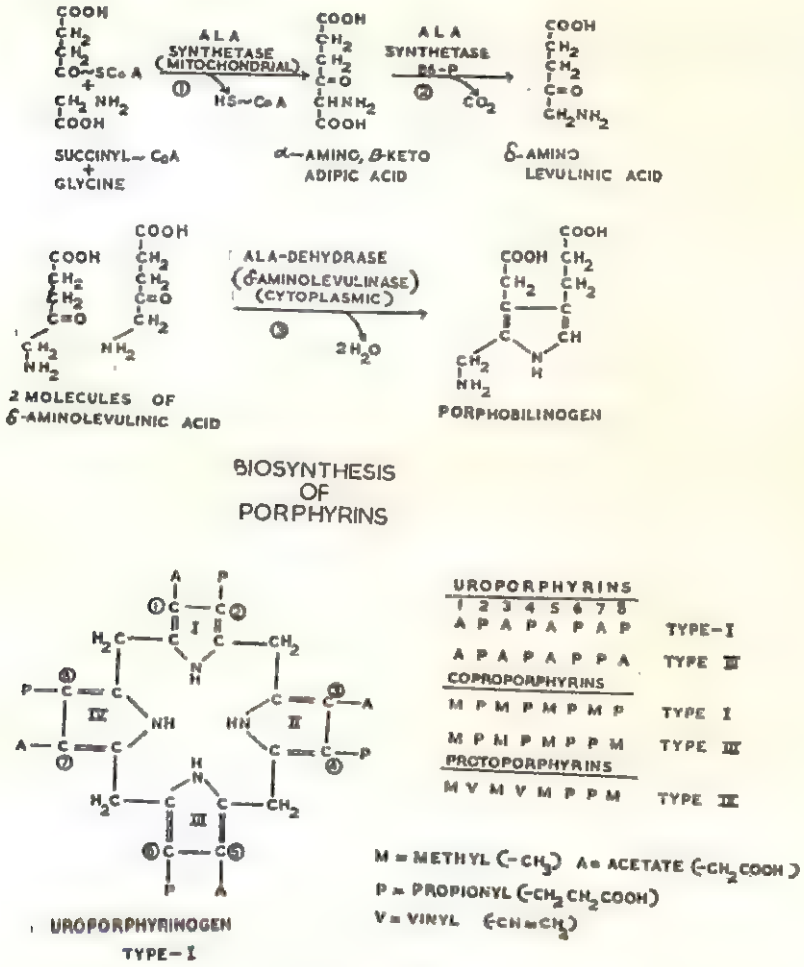


Fig. 19-1. Biosynthesis of porphyrins

The enzyme coproporphyrinogen oxidase can act only on the type III coproporphyrins. The resulting tetrapyrrole is a protoporphyrin called type IX.

Introduction of ferrous iron into the centre of the type IX protoporphyrin by *'heme synthetase'* (also called *'ferrochelatase')* converts it into heme.

Synthesis of hemoglobin: This is restricted in adult man to the erythrocyte precursors of bone marrow. Protoporphyrin type IX, iron and the protein globin are required. Pantothenic acid, pyridoxal phosphate, folic acid, B_{12}, traces of copper and the intrinsic factor of Castle are all required. Pantothenic acid is required as a constituent of coenzyme-A and pyridoxal phosphate for the activation of ALA synthetase; B_{12} and folic acid are required probably for the intense nucleic acid synthesis associated with the rapid cell division in erythropoiesis. Copper may be required for some of the oxidation reactions.

A low moclecular weight glycoprotein 'erythropoietin' produced in the kidney stimulates the formation, maturation and release of erythrocytes by bone marrow.

The early stages of erythropoietic cells contain porphyrin. During the course or their development (maturation), the porphyrin is converted to heme by addition of iron and then to hemoglobin by addition of the protein, globin. The hemoglobin once formed in the erythrocyte does not participate in the dynamic state like other components. It persists for the life of the RBC (120 days) and is liberated only on disruption of the R.B.C.

About 8 grams of hemoglobin are synthesized and degraded per day, requiring 300 mg of porphyrin a day and releasing 300 mg of bile pigments.

General properties of porphyrins: On account of the -COOH groups of side chains (propionic and acetic) and on account of -NH groups of the pyrrole rings, they act as weak acids and weak bases (amphoteric) and have isoelectric pH varying form 3.0 to 4.5. The porphyrinogens are colorless. Porphyrins are colored.

All porphyrins show a powerful absorption band at 400 nm. This is called the *'Soret band'* after its discoverer. The metalloporphyrins like heme have their own absorption spectra which differ from that of the parent porphyrin.

The metalloporphyrins can combine with proteins to form the various biologically important substances like hemoglobin and cytochromes. They can also combine with nitrogenous bases like pyridine to form hemochromogens.

Porphyrinuria: Normally only small amounts of coproporphyrins of types I and III (60–280 microgrammes in the ratio of 70% type I and 30% type III) are excreted in urine per day. Uroporphyrins are excreted only in negligible amounts of 15 to 30 microgrammes a day. In conditions like fever, poisoning by heavy metals, blood dyscrasias, diseases of the liver and pancreas and malignant conditions, coproporphyrins may be excreted in much larger amounts.

The feces normally contain 300–1000 microgrammes of coproporphyrins per day, mostly of the type I.

Porphyrias:

About 15% of heme synthesis occurs in the liver and 85% in the erythrocyte precursors. Hence, porphyrias can be of two types — hepatic and erythropoietic Many of the hepatic porphyrias and all erythropoetic porphyrias are inherited.

Erythropoietic protoporphyria: Inherited as autosomal dominant. The enzyme *'ferrochelatase'* which adds iron to protoporphyrin IX is deficient. Protoporphyrin IX accumulates in erythrocytes and plasma and is excreted in feces in large amounts. Anemia and cutaneous hypersensitivity to light are the main features.

Acute intermittent porphyria: Also inherited as autosomal dominant. The enzyme *'uroporphyrinogen I synthase'* is deficient in the liver and the erythropoetic cells. Urine contains porphobilinogen and δ'-aminolevulinate. The urine develops wine-red color due to oxidation of porphobilinogen on exposure to light. Barbiturates, alcohol, chloramphenicol and steroid hormones aggravate the condition by stimulating δ -aminolevulinate synthetase and increasing thereby the synthesis of porphobilinogen. Abdominal pain, vomiting, constipation, paralysis and psychological changes are some of the features of the condition.

Hereditary coproporphyria: Autosomal dominant inheritance. It is caused by deficiency of *'coproporphyrinogen synthetase'* in the liver. Delta-aminolevulinate and porphobilinogen accumulate. It manifests cutaneous hypersensitivity to light in addition to the other symptoms of the acute intermittent porphyria. Urine contains coproporphyrinogen, type III.

Porphyria cutanea tarda: This is the commonest form of porphyrias. *'Uroporphyrinogen decarboxylase* acitivity is deficient in the liver as well as the erythropoietic cells. Uroporphyrinogen III is not converted to coproporphyrinogen III. Large amounts of uroporphyrinogens and uroporphyrins are excreted in urine. Urine is colored pink or brown. There is hepatic damage and accumulation of iron in that viscera. There is photosensitivity of skin. The disease is acquired in alcoholism and other conditions causing liver damage. There is probably an increased susceptibilty in these individuals to a deficiency of the uroporphyrinogen decarboxylase activity.

Lead poisoning: Lead inhibits the enzyme *'uroporphyrinogen I synthetase'* (by displacing the zinc present in the enzyme) and the ferrochelatase enzyme. As a result, protoporphyrin IX and its zinc derivative accumulate. Anemia, abdominal pain and neurological symptoms occur.

The catabolism of heme and the formation and excretion of bile pigments have already been considered.

References:

Bishop, C. and Surgenor, D.M.: The Red Blood Cell., Acad. Pr., N.Y., 1964.

Chance, B. Estabrook, R.W. and Yonetani, T. (eds): Hemes and Hemoproteins: Acad. Pr., N.Y., 1966-68.

Falk, J.E.: Porphyrins and Metalloporphyrins. American Elsevier, N.Y., 1964.

Lascelles, J.: Tetrapyrrole Biosynthesis and its Regulation, Benzamin, N.Y., 1964.

Sunderman, F.W. and Sunderman, F.W. (Jr): Hemoglobin, Its Precursors and Metabolites., Lippincot Philadelphia, 1964.

20

WATER AND ELECTROLYTE BALANCE

THE water content of the human body varies from 50 to 70%, averaging about 55% in adult man and 50% in adult woman. It is distributed between two main compartments, the extracellular and the intracellular.

These can be further sub-divided thus:

In a 70 kg adult man	*% of total body weight*	*Amount*
A. Intracellular water	50%	35 litres.
B. Extracellular	20%	14 litres.
(i) Plasma	5%	3.5 litres
(ii) Interstitial fluid	15%	10.5 litres

The extracellular fluid includes plasma, interstital fluid, C.S.F., occular fluid, lymph, peritoneal, pericardial, pleural and synovial fluids. Of the interstital fluid, about a half is not readily accessible and does not take part in quick exchanges with the rest of the body water. This fraction includes the water contained in dense connective tissue, cartilage and bone and accounts for about 4.0 litres. The rest of interstitial fluid (6.5 litres) and plasma (3.5 litres) constitute the active or mobile part of the extracellular compartment.

Determination of fluid compartments:

Total body water: *Direct method.* The body is weighed, animal sacrificed and dehydrated completely and weighed again.

Indirect method: This is determined by injecting a known amount of a substance that readily permeates all fluid compartments and after allowing time for equilibration, determining its concentration in plasma. Deuterium oxide (D_2O), tritium oxide (T_2O) and antipyrine are some useful substances.

Extracellular fluid: This is determined by administering a substance which can penetrate all extracellular spaces, but is inpermeable to the cell membrane and which is not metabolized by the tissues. Inulin and mannitol are suitable for the purpose. The thiosulfate space also represents the extracellular fluid volume.

Intracellular fluid: This is obtained by difference between the total body water and extracellular water.

Plasma volume: This is determined by using a dye like Evans blue (T-1824) or I^{131} labelled albumin which is impermeable (or slowly permeable) during the short period of experiment.

Interstitial fluid Volume: Is obtained by difference between the extracellular fluid and plasma volumes.

The body water is maintained within fairly constant limits by a regulation between the intake and output as follows:–

Daily input		*Daily output*	
Plain water and beverages	1000–1500 ml.	Urine	1000–1500 ml.
Water used in cooking	700 ml.	Perspiration	400 ml.
		Feces	200 ml.
Water derived during metabolism of food	300 ml.	Respiration	400 ml.
	2000–2500 ml.		2000–2500 ml.

All the factors of input as well as output are highly variable. Thus the intake may be much more in the form of water and beverages in the hot months of the year. This is balanced by excessive perspiration and respiratory losses.

Control mechanisms: The intake is regulated by the mechanism of thirst. A 'thirst centre' located in the 3rd ventricle regulates the amount of water consumed as water or beverages. A deficient intake of water leads to concentration of the body fluids (with respect to the solutes) and a rise in their osmotic pressure. This tends to draw out water from the intracellular compartment. This dehydration of the cells seems to be the main stimulus for thirst mechanism through osmoreceptors as well as sensory nerves of the mouth and pharynx (glossopharyngeal and vagus) which respond to the dryness of the mouth and pharynx.

When there is more water in the fluid compartments, there is lowering of the osmotic pressure of the fluids. This causes a suppression of the production or release of the antidiuretic hormone by the posterior pituitary and a decrease in the reabsorption of water by the distal convoluted tubule and thus promotes diuresis and loss of body water.

Dehydration: As a result of water deprivation, concentration occurs first in the plasma, soon to be followed by the other extracellular compartments and later the intracellular compartment. Along with water, intracellular potassium also passes out into the extracellular fluid. This is to some extent compensated by passage of sodium from extracellular fluid into the cell. Deprivation of 20% of body water results in death, particularly if the deprivation is rapid.

Causes of Dehydration:

1. Non-availability of water as in shipwrecked sailors or persons lost in a desert. If the former drink seawater, it worsens the condition due to excretion of a large volume of urine to remove excess electrolytes ingested with sea water.

2. Difficulty in swallowing, unconsciousness and impairment of the sensation of thirst.

3. Diabetes insipidus and diabetes mellitus.

4. Chronic nephritis due to inability of tubule to concentrate urine.

5. Severe diarrhoea and vomiting.

6. Excessive perspiration and loss of fluids from skin in burns.

7. Excessive loss of water through respiration in prolonged exposure to sun (heat stroke).

In conditions of dehydration, the tongue is dry, skin hot and dry, and there is very little of saliva or tears. The packed cell volume which is normally 47±7% in men and 42±5% in women is increased. Hemoglobin concentration and plasma protein concentration are also increased. Plasma electrolytes are increased and urinary output is much diminished. Plasma non-protein nitrogen and urea concentrations are therefore increased.

The condition has to be remedied by giving plenty of plain water to drink and intravenous infusions of isotonic saline with or without glucose.

Water Intoxication:

Thic can occur due to:

1. Hypersecretion of ADH following the administration of an anaesthetic for surgery, administration of narcotic drugs like morphia and in stress due to any cause including that due to surgery. There is usually a period of water retention for 12 to 36 hours following a surgical operation.

2. Renal failure due to any cause can lead to water retention.

3. Excessive administration of fluids by mouth or parenterally.

The subject becomes mentally confused, develops aphasia, incoordination, delirium, muscular weakness, nausea and vomiting. He may finally develop convulsions and coma.

The packed cell volume, hemoglobin concentration and plasma protein concentration are all diminished. Urine volume is usually increased and is of low specific gravity. Plasma electrolytes are lowered.

The condition is treated by withholding fluids by mouth and administering 3–5% hypertonic saline intravenously.

Water Metabolism in Infants:

The body of an infant contains about 80% water compared to only 70% in the adult. Of this the ECF forms about 20%. In a 7.0 kg. infant, the ECF amounts to about 1.4 litres. Since his diet is mainly liquid, his input and output per day will be 0.7 litres, which is 50% of the ECF volume. (In an adult who has an ECF volume of 14 litres the input and output are only 2.0 to 2.5 litres, which amounts to between 14-16% of ECF volume only). Hence the fluid balance in the infant has to be carefully maintained since any deficit in intake or excess in output will cause immediate shrinkage in ECF volume with all the consequences of dehydration.

Electrolyte balance

Na^+, K^+, Cl^- and HCO_3^-, form the chief ions of the body fluids. The approximate distribution of the electrolytes in a 70 kg. man is shown in table 20-1.

TABLE 20-1.

Volume and composition of blood plasma, gastrointestinal secretions, and sweat.*

Fluid	Average Volume (ml/24 hours)	Electrolyte Concentrations (mEq-liter) Na^+	K^+	Cl^-	HCO_3^-
Blood plasma		135-150	3.6-5.5	100-105	24.6-28.8
Gastric juice	2500	31-90	4.3-12	52-124	0
Bile	700-1000	134-156	3.9-6.3	83-110	38
Pancreatic juice	>1000	113-153	2.6-7.4	54-95	110
Small bowel (Miller-Abbott suction)	3000	72-120	3.5-6.8	69-127	30
Ileostomy					
Recent	100-4000	112-142	4.5-14	93-122	30
Adapted	110-500	50	3	20	15-30
Cecostomy	100-3000	48-116	11.1-28.3	35-70	15
Feces	100	<10	<10	<15	<15
Sweat	500-4000	30-70	0-5	30-70	0

*Lockwood & Rendell: Bul. New York Acad Med 25:228, 1949; and Randall, S. Clin North America 32:3, 1952.

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In expressing electrolyte concentration, it is advantageous to indicate the concentration in terms of milliequivalents per litre instead of milligrammes per 100 ml. The body fluids maintain electrical neutrality–the anions balancing the cations. To get the anion and cation concentration, it is necessary to know the electrolyte concentration as milliequivalents. It can be readily calculated thus–

$$\text{Concentration in m.eq./litre} = \frac{\text{mg/100 ml} \times 10}{\text{equivalent weight}}$$

For sodium it is $\frac{327 \times 10}{23}$ i.e., 142 m.eq./litre.

The extracellular fluid: The composition of the extracellular fluid (ECF) has a remarkable resemblance to the composition of sea water of the Precambrian era at which time animals with closed circulation were evolved. The concentration of solutes has since increased in the sea water, but has remained the same in the ECF.

Osmotic Pressure of Body Fluids:

The osmotic pressure of a solution depends on the number of particles dissolved in it. In the case of substances which do not dissociate in solution (eg. glucose), the molar concentration of the solution will be an index of the number of particles in solution. In case of substances which dissociate in solution (eg. most of the electrolytes like NaCl $NaCl \rightarrow Na^+ + Cl^-$), each molecule of the substance is equivalent to two particles. In terms of osmotic pressure, it is equal to 2 molecules of an undissociated substance. To distinguish the molar concentration from the concentration of osmotically effective particles, the concept of *'osmolar concentration'* is developed. A molar solution of undissociated solute has an osmolar concentration of I. A molar solution of a completely dissociated substance like NaCl has an osmolar concentration of 2. For Na_2HPO_4, it will be 3 ($Na^+ + Na^+ + HPO_4^=$).

As in all other biological measurements, since concentrations are much smaller than molar, they are expressed as millimols and milliosmols per litre.

Plasma electrolyte composition:

Cations.m.eqs./litre			*Anions.m.eqs/litre*		
Na^+	..	142	Cl^-	..	103
K^+	..	5	HCO_3^-	..	27
Ca^{++}	..	5	$HPO_4^=$	..	2
Mg^{++}	..	3	$SO_4^=$	..	1
			Org. acids	..	6
		155	Protein	..	16
					155

The extracellular fluid in man has a concentration of 310 milliosmols per litre (m.Osm/L) which is the sum total of 155 m.eqs. of cations and 155 m.eqs. of anions.

Electrolyte composition of other body fluids: Except that the protein concentration is much lower, the other constituents are similar.

Electrolyte composition of tissue fluids (intracellular): Taking muscle as a typical and the most extensive of all the tissues, the composition of its cell fluid is as follows–

Cations.m.eqs./litre			*Anions.m.eqs/litre*		
Na^+	..	10	HCO_3^-	..	8
K^+	..	148	Phosphates & others	..	136
Ca^{++}	..	2	Protein	..	56
Mg^{++}	..	40			
Total:		200			200

The differences are readily obvious. The chief cations of intracellular fluid are K^+ and Mg^{++} and these are balanced mainly by the chief anions, phosphate and protein. Chloride and sodium and bicarbonate are present only in minimal amounts. Total electrolyte concentration is higher than in extracellular fluids. The differences are clearly seen in fig. 20-1.

The phosphates of the cell are the phosphoric esters of hexoses, creatine phosphate and ATP, and inorganic phosphate.

The higher concentration of the non-diffusible protein anion in the cell compared to its counterpart in the extracellular fluid gives rise to a Donnan effect on the diffusible electrolytes and is responsible for their different concentrations in the extracellular and intracellular fluids.

Role of solutes in maintaining the volumes of the fluid compartments:

Electrolytes, especially sodium and potassium: Sodium is the backbone of the extracellular fluid and mainly determines the E.C.F. volume. Potassium is the main intracellular fluid electrolyte. Water is freely diffusible between the different compartments. Its volume in the different compartments is osmotically maintained by the electrolyte content primarily. The potassium of the cells may pass into the extracellular compartment in conditions of severe potassium loss through vomiting or diarrhoea. If the fluid loss is replaced by fluids containing only sodium, some of the sodium may enter the cells to replace the potassium loss.

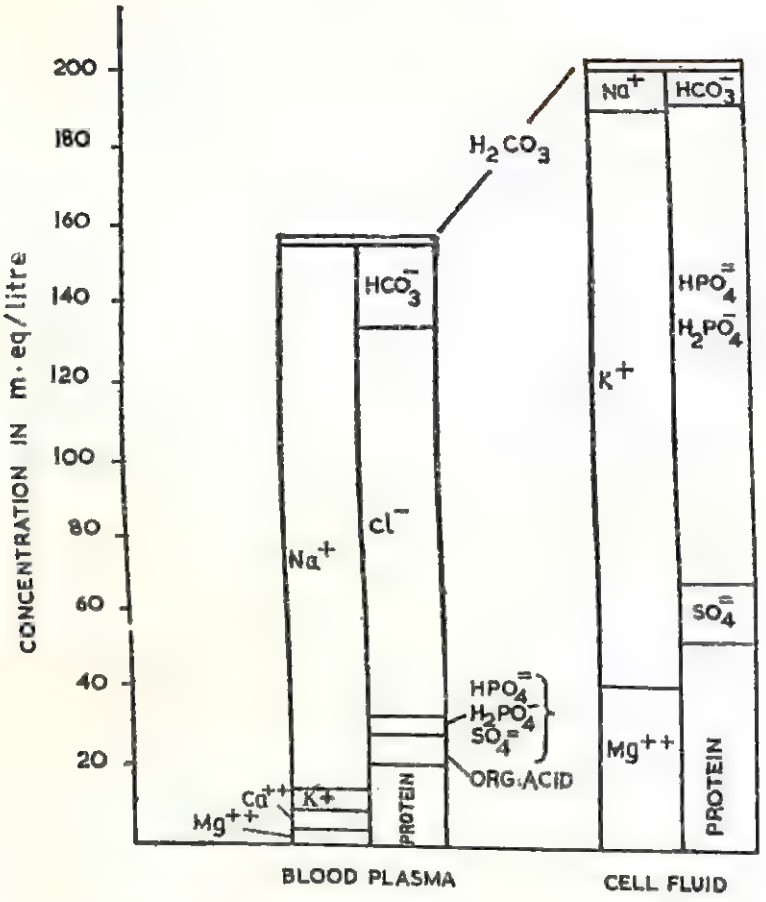
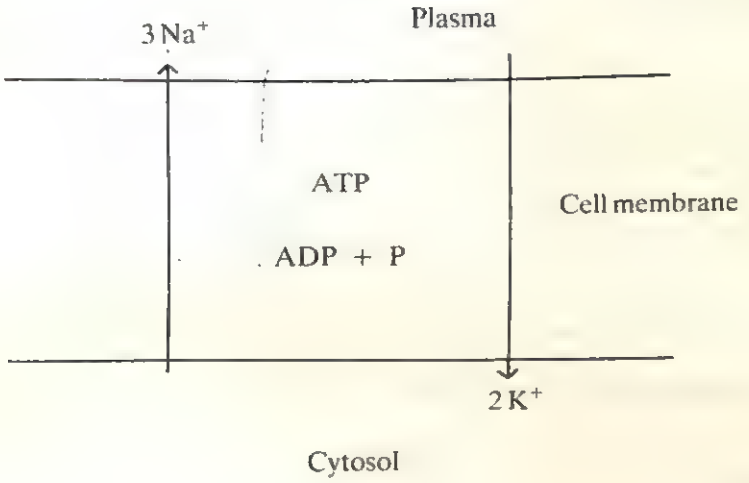


Fig. 20-1. Electrolyte composition of blood plasma and intracellular fluid.

The sodium-potassium pump: An energy-requiring pumping mechanism maintains the high gradient of Na^+ and K^+ on either side of the cell membrane. The mechanism involves the enzyme 'Na^+, K^+-ATPase' The energy derived from the breakdown of ATP is utilized to maintain the ionic concentrations of the intra and extracellular fluids against gradient. The Na^+ of ECF is several times that of intracellular fluid (ICF). Similarly, the concentration of K^+ of ICF is several times that of ECF.

The Na^+, K^+-ATPase enzyme is a tetramer and has two alfa and two beta subunits – $\alpha_2\beta_2$. It is associated with phospholipid and carbohydrate and requires Mg^{++} ions for its activity **By the action of the enzyme one molecule of ATP breaks down to an ADP and phosphate, with the simultaneous pumping of 3 Na^+ from inside the cell and the pumping in of 2 K^+ into the cell, both processes against a concentration gradient.**



Not only a chemical potential is maintained, but in many cases (eg.nerve cell, skeletal muscle) an electric potential is also maintained, the inner surface of the membrane being 50-90 m.v. electronegative with respect to the outer surface.

The mechanism of action of this pump is not clear. The enzyme extends through the entire thickness of the membrane. One of the aspartate residues near the inner wall of the membrane seems to take up the inorganic phosphate from the ATP to form a transient anhydride with one of its carboxylic groups. This is high energy bond. The enzyme molecule somehow takes up three Na^+ ions and then undergoes a conformational change leading to an exposure of the 3 Na^+ to the outer surface of the membrane where they are released into the ECF and simultaneously 2 K^+ are taken. The enzyme molecule now reverts back to its original conformation drawing the 2 K^+ into the cytosol and releasing the phosphate from aspartate.

Flow in the reverse direction of the pump: The chemical and electric potential gradients whose maintenance requires energy can, in turn, help in driving certain other processes. The flow of soidum back into the cell and potassium out of the cell are along gradients, but do not occur by passive diffusion. The transport is facilitated by carrier molecules (transport proteins). The transport is also aided by conformational changes in the membrane proteins resulting in an opening up of previously closed or previously narrow channels. If this flux of ions occurs suddenly as in the nerve cell, it results in depolarization of the cell membrane and generation of a nervous impluse. In other cases, it may help in the transport of other substances like glucose against gradient. The carrier proteins for glucose undergo conformational changes when combined with Na^+ (from intestinal lumen, say) and this results in both sodium and glucose facing inwards into the cell. They are both released into the cytoplasm. This is an example of *"Symport"*, transport of two components in the same direction.

The excess sodium is later pumped out into the blood by the Na^+, K^+-ATPase present on the membrane on the serosal side of the intestinal mucosal cell.

The reverse occurs in "*Antiport*" where the movemnent of a component in one direction drives another component in the reverse direction. In either case, one of the components is moving from a higher to a lower concentration gradient and this helps drive a second component from a lower to a higher concentration gradient.

This principle is made use of in the treatment of cholera. In the severe diarrhoea associated with it, large amounts of electrolytes (mainly Na^+) are lost in the diarrhoeal fluid. This can be remedied by continuous infusion of glucose through a tube into the intestines. As glucose is absorbed, sodium also gets absorbed.

Control of Total Electrolytes and Osmotic Pressure:

The ECF is the fluid compartment in circulation throughout the body and any alterations of the electrolyte composition of any other fluid compartment are ultimately passed on to the ECF. Regulation of the electrolyte composition and osmolarity of the different fluid compartments is therefore best brought about by altering the composition of the ECF. The kideny is the main regulator of the ECF composition, under the influence of the ADH and the mineralocorticoids.

ECF volume: Plasma volume is mainly dependent on the concentration of plasma protiens which retain the appropriate amount of fluid in the vascular system due to the colloidal osmotic pressure exerted by them, and prevent its passage into the interstitial fluid space.

The total ECF volume, on the other hand, is dependent on the total sodium content of the body and varies directly as the sodium content. If hypertonic saline is ingested or injected intravenously, due to increased osmolarity of the plasma, intracellular fluid comes out of the cells to dilute the ECF. There is hence a dehydration of the tissues. This is made use of in the treatment of edema of the brain, a serious condition. Hypertonic saline is injected intravenously to draw away the edema water from the brain.

Administration of isotonic saline, on the other hand, causes an expansion of the ECF volume and the kideny takes quite some days to excrete all the excess salt and restore the volume to normal. This is because the kideny is less sensitive to alterations in ECF volume and reacts sluggishly. It reacts much quicker and more efficiently to change in the osmolarity of the ECF.

On low salt diets, the kideny conserves sodium and chloride by excreting less of them. Since the total body fluid depends on body sodium, diminished intake of sodium will finally result in diminished ECF volume. Low sodium diets are hence prescribed in congestive heart failure to decrease the ECF volume and thereby reduce the load on the heart.

Variations in ECF Volume and Electrolytes:

Changes in ECF volume are indicated by changes in the hematocrit reading, hemoglobin and plasma protein concentrations. An expansion (increase) of ECF volume lowers all the

three. A concentration (decrease) in ECF volume, on the other hand, causes an increase in all the three. The expansion and contraction of the ECF volume can be hypertonic, isotonic or hypotonic, thus giving rise to six different conditions.

1. *Isotonic expansion:* It is usually due to administration of excessive amounts of normal saline. Edema may occur in the lungs and extremities; there is no passage of tissue fluids outside. Compensation is brought about by increased elimination of salt and fluid by kidney. Serum sodium levels are normal.

2. *Hypertonic expansion:* This is due to drinking hypertonic salt solutions (e.g. sea water). Due to hypertonicity of ECF, the cell water is drawn out, causing a dehydration of the tissues. Kidney attempts compensation by excreting more of salt and water. Serum sodium levels are high.

3. *Hypotonic expansion:* This can arise out of ingesting large amounts of plain water or injection of dextrose solution without salt. The water will pass into the tissues (intracellular compartment) to maintain osmotic balance between the two compartments. Kidney attempts to compensate by excretion of more water and less salt. Serum sodium levels are low.

4. *Isotonic contraction:* This occurs in conditions like diarrhoea, vomiting and hemorrhage, where isotonic fluids are lost. Due to decrease in ECF volume, there is circulatory failure, fall of blood pressure and renal failure. Blood urea and non-protein nitrogen are increased. The intracellular fluid remains unaltered. Kidney attempts compensation by diminished excretion of both water and salt.

5. *Hypertonic contraction:* Diminished water intake due to any cause can bring this about. The diminished intake may be due to non-availability of water (as in shipwrecked sailors or castaways in desert); it may be due to illness or unconsciousness; it may also occur due to excessive loss of salt free (compared to the concentration in plasma) fluid in sweating (as in sunstroke) or in urine (as in diabetes mellitus or insipidus). Intracellular water passes out into the ECF to compensate the hypertonicity of the latter. Most of the symptoms are on account of this dehydration of tissues, particularly the C.N.S. Kidney attempts compensation by increased excretion of salt with less of water. Serum sodium concentration is increased.

6. *Hypotonic contraction:* More salt is lost from the body than water. This can occur in Addison's disease, certain types of brain injury and chronic renal tubular dysfunction with impairment of ability to reabsorb sodium. Both water and salt are lost, but more of salt. To bring about osmotic equilibrium, water from ECF passes into cell, causing further contraction of ECF volume, circulatory failure and renal failure. Serum sodium concentration is low.

Regulatory mechanisms:

1. *ADH secretion:* The antidiuretic hormone (ADH or vasopressin) is released from the neurohypophysis and acts on the distal convoluted tubules of the kidney to cause increased water reabsorption and diminished urine volume (antidiuresis). The stiumulus for its release is an increased electrolyte content i.e., increased osmotic pressure of the plasma, which stimulates osmoreceptors in the diencephalon, which pass on the stimuli to paraventricular and supraoptic nucleii to produce more of ADH. The ADH is passed on to neurohypophysis and released from there. The retention of water enables plasma to be diluted and brings down the osmotic pressure to normal levels.

The release of ADH is suppressed when the plasma electrolyte concentration and osmotic pressure are lower than normal. Hence tubular reabsorption of water is diminished, diuresis occurs and plasma becomes concentrated.

2. *Mineralocorticoids:* Hormones of the adrenal cortex which do not have a 'O' or 'OH' in position 11 (e.g. deoxycorticosterone) and aldosterone particularly exert a potent effect on the mineral metabolism, specifically of sodium and potassium. When plasma osmotic pressure is lowered, the adrenal cortex is stimulated to secrete the mineralocorticoids which act on the distal convoluted tubules of the kidney to increase the reabsorption of sodium salts, thus restoring the osmotic pressure.

The secretion of the hormones is suppressed when there is an elevated osmotic pressure, thus allowing more of sodium salts to be excreted by the kidney to bring down the osmotic pressure.

3. *The mechanism of thirst:* This has been already referred to under water balance.

In general, the volume of intracellular fluid is dependent on the potassium content of the cell and the volume of extracellular fluid on its sodium content. Any fluid ingested or administered parenterally without salts is promptly excreted in urine whereas isotonic saline is retained for much longer periods even though the total fluid volume is increased. Conversely, administration of hypertonic saline results in prompt excretion of the sodium in isotonic solution i.e., enough water to make the solution isotonic is lost from the body resulting in dehydration.

Volume receptors: Increases in the volume of circulating blood plasma itself may stimulate mechanisms for the elimination of more fluid by the kidney. These mechanisms operate through pressure receptors (baroreceptors) and volume receptors (or *stretch receptors*) which are located in the large veins near the heart and in the right atrium.

References:

Bland, J.H. (ed): Clinical Metabolism of Body Water and Electrolytes; Saunders, Philadelphia; 1963.

Elkinton, J.R. and Danowski, T.S.: The Body Fluids; Williams and Wilkins, Baltimore, 1955.

Gamble, J.L.: Chemical Anatomy. Physiology and Pathology of Extracellular Fluids, Harvard Univ. Pr., Cambridge (Mass)., 1954.

Goldberger, E.: A Primer of Water, Electrolytes and Acid-Base Syndromes, 4th ed., Lea and Febiger, 1970.
Maxwell, M.H. and Kleeman, C.R.: Clinical Distrubances of Fluid and Electrolyte Metabolism, McGraw-Hill, N.Y., 1962.
Pitts, F.: Physiology of Kidney and Body Fluids, Yearbook Med. Pub. Chicago, 1963.
Weisberg, H.F.: Water, Electrolyte and Acid-Base Balance, 2nd ed., Williams and Wilkins, Baltimore, 1962.

21

CHEMISTRY OF RESPIRATION

AN average adult man at rest utilizes about 250 ml. of oxygen and eliminates 200 ml. of carbondioxide per minute. The quantities increase ten fold during strenuous exercise. The supply of the required amount of oxygen to the tissues and the elimination of CO_2 are both brought about by blood. The arterial blood supplies oxygen to the tissues and takes up carbondioxide from them and becomes converted to venous blood. This, in turn, passes into the lungs where it gives up the carbondioxide and takes up oxygen from the alveolar air to be reconverted to arterial blood. The average gaseous composition of arterial and venous blood is given in Table 21-1.

TABLE 21-1.

	O_2		CO_2		N_2	
	ml/100 ml.	pO_2 *mm.Hg.*	*ml/100 ml.*	pCO_2 *mm.Hg.*	*ml/100 ml.*	pN_2 *mm.Hg.*
Inspired air	20.9	158	0.04	0.3	79.0	597
Alveolar air	14.2	100	5.6	40	80.3	570
Arterial blood	19.6	100	48.2	40	0.9	570
Venous blood	12.6	40	54.8	46	0.9	570
Interstitial fluid		30				
Tissue fluid		10		50		

The aqueous tension in alveolar air is about 48mm. Hg.

Transport of oxygen: The venous blood, as it enters the lungs, contains 12.6 ml. oxygen at a partial pressure (conventionally referred to as pO_2) of 40 mm. of Hg. It is separated from the alveolar air by only a thin endothelial lining of the capillaries and alveoli (not more than 1-2μ thick). The alveolar air contains oxygen at a pO_2 of 100 mm. This enables oxygen to rapidly diffuse through the thin membrane into the blood, first in physical solution into

plasma, later to be taken up by the hemoglobin of the erythrocyte. The total surface of the alveoli is so large (50 to 100 sq. meters) that during the short period of about 0.7 seconds the blood takes to traverse the lung capillaries, it attains equilibrium with alveolar air and becomes the arterial blood.

Arterial blood contains an average of 19.6 ml/100 ml. at a pO_2 of 90 mm of Hg. At this pressure only 0.3 ml of oxygen is in physical solution. The rest is present in loose combination with hemoglobin as oxyhemoglobin.

$$Hb + O_2 \longleftrightarrow HbO_2$$

Hemoglobin is 97% saturated with oxygen under these conditions. The arterial blood now passes to the tissues and as it enters the capillary bed of the tissues, it is again separated only by a thin endothelial lining of the capillaries from the interstitial fluid, where the pO_2 is only 30 mm. The O_2 in physical solution rapidly diffuses out first and is followed by O_2 released by the dissociation of HbO_2.

$$HbO_2 \longleftrightarrow Hb + O_2$$

By the time the blood traverses the capillary bed and becomes venous blood, it has lost about 7.0 ml of oxygen and the pO_2 falls to 40 mm. The hemoglobin is only 70% oxygenated.

These changes involved in oxygen transport are closely interconnected with changes involved in CO_2 transport and resulting pH changes in blood (See fig. 6-2).

Oxygen Dissociation Curves

When a solution of myoglobin (which contains only one heme per molecule) is exposed to pure oxygen and the per cent saturation of the solution with oxygen is measured, the relationship between oxygen tension and per cent saturation of myoglobin gives a rectangular hyperbolic curve. At the O_2 tension of arterial blood (about 100 mm.Hg.) myoglobin is about 95% saturated. The resting capillary O_2 tension is about 40 mm.Hg. at the arterial end. In the active muscle, it may fall to as low as 20 mm. Hg. Even at such low oxygen tensions, myoglobin can be over 80% saturated with oxygen. The tissue oxygen tension during activity will be as low as 5 mm.Hg. and there is thus enough gradient for the myoglobin to supply oxygen to the tissues. It parts with about 12% of oxygen carried by it under these condtiions.

In the case of hemoglobin, there are four subunits in the molecule — $alfa_1$, $alfa_2$, $beta_1$ and $beta_2$ chains. The four subunits display a 'cooperative' effect in transporting oxygen. Each alfa unit is somewhat rigidly attached to a beta unit, but the attachment to the other beta unit is less rigid (i.e. the attachment of $alfa_1$ to $beta_2$ and $alfa_2$ to $beta_1$). Even these less rigid attachments show alterations between oxyhemoglobin and reduced hemoglobin. In the oxygenated state, these bonds are more relaxed (R state) and in the reduced state, these bonds are more taut (T state), due to breakdown of small salt bridges between the units in the R state, and their reformation in the T state. The $\alpha 2\ \beta 2$ dimer shows slight rotation

(about 15°) in relation to the $\alpha_1\beta_1$ dimer during the conversion of the R form to the T form. In the R form, Fe^{++} atom is exactly in the plane of the porphyrin ring. In the T form, it is 0.07 nm out of the plane. Also, a valine residue projects into the heme pocket of the beta chain in the T form and blocks the entry of oxygen. The valine residue is retracted away from the pocket in the R form due to the slight rotation and loosening of the salt bridges.

On account of all these factors, the R form has affinity for oxygen which is several hundred times that of the T form. Formation of the R form is favoured when one or two oxygen atoms occupy the heme pockets of the alfa units. Thus, the uptake of one or two oxygen atoms by the alfa units triggers the conversion of the T form to the R form and increases the affinity of the hemoglobin molecule to oxygen several hundred fold.

The reverse set of reactions occur when the molecule loses oxygen. The salt bridges are reformed, the $\alpha_2\beta_2$ unit rotates back to the preoxygenated state, valine projects back into the heme pockets of the beta chains and the affinity to oxygen is enormously reduced. Oxygen, therefore, rapidly dissociates from hemoglobin, and the reduced T form of hemoglobin is formed.

On account of this cooperativity in taking up oxygen by the four subunits of hemoglobin, the O_2 dissociation curves for hemoglobin assume an S-shape.

The salient differences between the T form and the R form of hemoglobin are presented in the table below.

	T (Taut) form (reduced Hb)	*R (relaxed) form (OxyHb)*
1.	$\alpha_1\beta_1$ and $\alpha_2\beta_2$ units have their long axis close	There is rotation of 15° between the two.
2.	Salt bridges are numerous.	Salt bridges less in number.
3.	Fe^{++} is 0.07 nm out of the plane of porphyrin ring.	Fe^{++} in the plane of porphyrin ring.
4.	Valine residue projects into heme pocket of beta chains.	Valine residue does not project. Heme pocket free to take up O_2.
5.	Affinity for O_2 is low.	Affinity for O_2 high by several hundred fold.
6.	Beta chain histidine residues are protonated (H^+ added).	Hidtidines of beta chains release protons ($2H^+$).
7.	DPG can enter and is retained by salt bridges in a central cavity formed between the four subunits.	DPG cannot bind.

Bohr Effect: The breaking of the salt bridges at the ends of the hemoglobin molecule on oxygenation releases the n-terminal valines of the alfa units and some of the histidines of the beta units. the pK values are lowered. At pH 7.0, there is a dissociation of protons from these amino acids on oxygenation.

$$HHb^{+} + O_2 \longleftrightarrow HbO_2 + H^{+}$$

This is known as the Bohr effect. Acidification of the hemoglobin molecule tends to stabilize the deoxy form of hemoglobin. Under conditions of low pO_2, high pCO_2 and lactic acid concentration in tissue capillaries, the lowering of pH causes oxyhemoglobin to release oxygen more efficiently.

Role of Diphosphoglycerate: This acts as an allosteric regulator of hemoglobin activity. It is present in the red cell in equimolar concentration to hemoglobin. The affinity of DPG to hemoglobin is much more (about two times as much) than for oxyhemoglobin. It binds allosterically hemoglobin molecules and causes conformational changes similar to oxygen binding at the active site and thus helps a more efficient release of oxygen in conditions obtaining in tissue capillaries. The DPG levels increase in erythrocytes of people living at high altitudes.

CO_2 itself acts as an allosteric regulator by combining reversibly with the $-NH_2$ of the n-terminal amino acids to form carbamine hemoglobin. Hemoglobin has a higher affinity for CO_2 than oxyhemoglobin. As the O_2 is lost from the oxyhemoglobin, CO_2 is taken up by the hemoglobin to form carbamino-hemoglobin.

Fetal respiration: The fetus is supplied oxygen from placental blood, the pO_2 of which is quite low. Still, the fetal hemoglobin (HbF) can draw oxygen from placental blood, because the O_2 dissociation curve of HbF is considerably shifted to the left compared to adult hemoglobin. This is on account of the low affinity of HbF to DPG. At a pO_2 of 30 mm Hg., while HbA is only 33% saturated with oxygen, HbF is 58% saturated.

Oxygen toxicity: Exposure to oxygen under high pressure causes toxic symptoms and may prove fatal. Pure oxygen is an irritant to the lungs and produces pulmonary edema. Oversaturation of hemoglobin with oxygen will prevent the transport of carbondioxide, particularly the isohydric phase. The –SH enzymes also get oxidized to the inactive –S–S– forms.

Carbondioxide transport: Carbondioxide in solution forms carbonic acid which has the effect of lowering the pH to the acid side. But actually there is only slight lowering of pH by 0.01 to 0.03 in the conversion of arterial to venous blood, though large amounts (6.6 ml/100 ml of blood) of CO_2 are added to the venous blood.

As arterial blood enters the capillary bed in the tissues, it contains 48.2 vols% of CO_2 at a pCO_2 of 40 mm. This is separated by endothelial lining of capillary from the interstitial fluid which has a pCO_2 of around 50 mm. This gradient is sufficient to permit CO_2 to diffuse from the tissue fluid into the capillary blood. The CO_2 is first taken up as dissolved CO_2, but it is soon dealt with by other chemical mechanisms. The CO_2 content rises to 54.8 vols/100 ml by the time it reaches the veins as venous blood.

In the lungs the reverse set of conditions prevail where the blood in the capillaries is exposed to alveolar air with a pCO_2 of 40 mm. Carbondioxide diffuses out into the alveoli by a reversal of the chemical processes through the stage of physical solution to part with the 6.6 ml gained, to restore the original CO_2 content and pCO_2 of arterial blood.

Mode of transport of CO_2:

1. *Physical solution:* The solubility of CO_2 in plasma and erythrocyte fluid at pCO_2 of 46 (venous blood) is 3.2 ml/100 ml and at pCO_2 of 40 (arterial blood), it is 2.7 ml/100 ml. This accounts for transport of about 0.5 ml of CO_2 which is 7–8% of the 6.6 ml of additional CO_2 transported by venous blood.

2. *Chemical transport:*

(i) *Transport by plasma proteins:* The CO_2 combines with water to form carbonic acid, which, in turn, dissociates to form the hydrogen and bicarbonate ions. The hydrogen ion is taken up by the protein buffers and the bicarbonate by the bases present in plasma.

$$H_2O + CO_2 \longleftrightarrow H_2CO_3 \longleftrightarrow H^+ + HCO_3^-$$
$$H^+ + \text{Protein}^- \longleftrightarrow \text{H.protein}$$
$$HCO_3^- + B^+ \longleftrightarrow B\ H\ CO_3$$

Other buffer systems like the phosphate may also take up the hydrogen ion.

This accounts for 4% of the CO_2 transport (4% of 6.6 ml) i.e., about 0.26 ml.

Carbonic anhydrase: This enzyme facilitates the formation of H_2CO_3 and its dissociation to form bicarbonate. It is present in the erythrocytes and accounts for the formation of more than 90% of the HCO_3^- in the R.B.C.

(ii) *Transport by hemoglobin of the erythrocytes:* This occurs by two methods:-

(a) Formation of carbaminohemoglobin: Some of the free amino groups of hemoglobin readily combine with CO_2 in a reversible reaction to form carbaminohemoglobin.

$$Hb\ NH_2 + CO_2 \longleftrightarrow Hb\ NH\ COO^-$$

Reduced hemoglobin has a greater affinity for CO_2 than oxyhemoglobin. Since the uptake of CO_2 and the release of O_2 go together, the conditions are highly favourable for carbaminohemoglobin formation. This accounts for 20% of the CO_2 transported (20% of 6.6 ml) i.e., 1.3 ml.

(b) *Isohydric transport of CO_2 by hemoglobin:* Quantitatively this is the most important and accounts for about 70% of the CO_2 transported (70% of 6.6 ml. i.e. 4.6 ml). Further, the transport is managed without the slightest change in pH on this account (hence isohydric).

Haldane effect: Binding of O_2 to hemoglobin displaces CO_2 from it. This is known as Haldane effect.

Both hemoglobin and oxyhemoglobin behave as weak acids. Between the two, oxyhemoglobin is a stronger acid ($pK=2.4\times10^{-7}$) and dissociates to give off more hydrogen ions than reduced hemoglobin ($pK=6.6\times10^{-9}$).

If the two hemoglobins are represented to indicate their acid nature as $HHbO_2$ and HHb, then the conversion of $HHbO_2$ to HHb which occurs during the passage of blood through the capillary bed of tissues will require addition of hydrogen ion to the weaker acid HHb formed. During this phase the reactions

$$H_2O + CO_2 \longrightarrow H_2CO_3 \longrightarrow H^+ + HCO_3^-$$

are occuring simultaneously due to intake of CO_2 from the tissues. The HHb will take up the H^+ ion and the HCO_3^- is taken up by the bases (K^+ mainly in the cell). This is known as the isohydric transport of CO_2.

Chloride-bicarbonate shift: Most of the CO_2 (90%) is thus taken up by the erythrocyte. It is reasonable to expect a rise in the bicarbonate content of erythrocyte in the venous blood compared to the arterial blood. But, in fact, it is the venous plasma which shows an increase in bicarbonate content. There is a corresponding decrease in the chloride content of the venous plasma and an increase in the erythrocyte chloride. So, obviously, the bicarbonate formed in the erythrocyte is passed out into the plasma and has been replaced by an equivalent amount of chloride from plasma. This can be explained on the principle of Donnan's membrane equilibrium.

	Erythrocyte	*Plasma*
Readily permeable	HCO_3^-	HCO_3^-
	CL^-	CL^-
	OH^-	OH^-
Impermeable	Hb^-	$Protein^-$
	HbO_2^-	
	H^+	H^+
	K^+	Na^+

The erythrocyte membrane is impermeable to the cations H^+, and Na^+ and K^+. It is also impermeable to the large protein molecules, including hemoglobin. It is however freely permeable to CL^-, $H\,CO_3^-$, and OH^- Electrical neutrality, as well as osmotic balance have to be maintained by a movement of the three ions only across the cell membrane as required.

The concentration of the nondiffusible Hb^- and HbO_2^- in the cell is much more than the protein of the plasma. This sets up a Donnan effect across the cell membrane. The diffusible anious HCO_3^-, CL^- and OH^- will hence be in higher concentration in the plasma than within the cell. At the same time the ratios

$$\frac{(H\,CO_3^-)\,\text{cell}}{(H\,CO_3^-)\,\text{plasma}} = \frac{(CL^-)\,\text{cell}}{(CL^-)\,\text{plasma}} = \frac{(OH^-)\,\text{cell}}{(OH^-)\,\text{plasma}}$$

During the passage of blood through tissues CO_2 is taken up by the cell to form $H\,CO_3^-$ in large amounts. This disturbs the equilibrium above by raising (HCO_3^-) cell. To restore the equilibrium the HCO_3^- moves out from the cell into plasma and CL^- and OH^- move into the cell from plasma.

The reverse set of changes occur in the lungs, where the bicarbonate of the cell is being rapidly lost by conversion to H_2CO_3. $HCO_3^- + H^+ \longrightarrow H_2CO_3$. The H^+ is released by conversion of the weak acid HHb to the stronger acid $HHbO_2$. The H_2CO_3 dissociates to form $H_2O + CO_2$. The CO_2 diffuses out into alveoli and is expired. There is thus a movement of the bicarbonate into the cell and chloride moves out into the plasma.

Significance of N_2 in respiration: There is no chemical mode of transport of nitrogen in blood. It is present only in physical solution. At the pN_2 of 570 mm 0.9 ml of nitrogen is dissolved per 100 ml. This remains the same in arterial and venous blood and in tissues. When the pressure is increased as in diving under the sea, more nitrogen is dissolved.

If in such an individual, there is a sudden decompression, the dissolved nitrogen will be liberated as bubbles of nitrogen gas into the blood and tissues. In blood this may cause embolism. In tissues, particularly nervous tissues, it will cause severe pain due to pressure on the nerves. The individual has to be again kept in a compressed atmosphere and decompression should be done in a gradual manner to allow time for the nitrogen to be swept out through respiration.

Control of respiration:

Respiratory centre: This is located in the medulla oblongata and sends out impulses to the respiratory apparatus. Increase in pCO_2 and H^+ ion concentration stimulate the respiratory centre directly. The rate and depth of respiration are increased till the excess CO_2 is blown out, thus allowing the pCO_2 and H^+ to return to normal. Decrease in pCO_2 and H^+ ion concentration depresses the centre and acts in a reverse way to conserve CO_2 and raise the H^+ ion concentration.

Chemoreceptors of the carotid and aortic bodies: These are stimulated by a decrease in pO_2 and increase in pCO_2 and H^+ ion concentration of the arterial blood. The impluses are carried through nerves to the respiratory centre which is reflexly stimulated. The main stimulus for chemoreceptors is pO_2 decrease.

Certain perfluoro compounds like *perfluorodecalin* can dissolve gases like O_2 and CO_2 proportional to their partial pressures. The dissociation curve is linear. They are also non-toxic, when used for short periods. Hence, in conditions which require partial or complete replacement of erythrocytes, emulsions of this compound are injected intravenously to temporarily serve the function of respiratory exchange of gases.

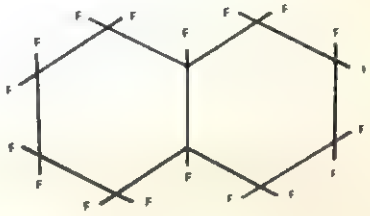


Fig. 21-1. Perfluorodecalin

References:

Barcroft J.S.: The Respiratory Function of the Blood, II, Hemoglobin, Cambridge Univ. Pr., London 1928.
Chance, B., Estabrook, R.W. and Yonetani, T. (eds): Hemes and Hemoproteins., Acad Pr., N.Y., 1967.
Ferguson, J.H.: Blood and Body Functions: Davis, Philadelphia, 1965.
Haldane, J.S. and Priestley, J.J.: Respiration, Yale Univ. Pr., New Haven, 1935.
Henderson, L.J.: Blood, a study in General Physiology., Yale Univ, Pr., New Haven, 1928.
Ingram, V.M.: The Hemoglobins in Genetics and Evolution., Columbia Univ, Pr. N.Y., 1963.
MacFarlane, R.G. and Robb-Smith, A.H.T.: Functions of the Blood., Acad., Pr., N.Y., 1961.

22

METABOLISM OF INORGANIC SUBSTANCES

THE metabolism of certain minerals like sodium and potassium is intimately connected with the water balance, osmotic pressure and pH regulation of the blood and body fluids. These aspects are dealt with in other chapters.

SODIUM, POTASSIUM AND CHLORIDE

In the excellular fluids, sodium is the chief cation and chloride an important anion while in the intracellular fluid, potassium is the chief cation. Potassium normally moves out of the cell only in small amounts. In severe dehydration of the cells, there may be a more rapid movement out of the cell. Sodium normally enters the cell but is rapidly removed by what is known as 'sodium pump', an energy requiring mechanism of transport from within the cell to extracellular fluid against gradient.

Sodium: Normal diets contain highly variable amounts from 5 to 15 grams (as sodium chloride) added during the preparation of food and this much is excreted in urine and sweat. 95% is excreted through urine. 5 grams of sodium chloride a day are probably adequate for a normal healthy adult. Hypertensives (where sodium in excess is harmful) can be maintained on amounts as low as 1 gram a day.

Sodium is present in bone as well as in soft tissues. But it is the sodium of the body fluids including blood which is metabolically of greatest importance. Whole blood contains 160 mg/100 ml and plasma contains 330 mg/100 ml. (143 m.eq/litre). Adrenal cortical hormones are required for the normal sodium metabolism. In their deficiency (eg: Addison's disease) there is an increase in the renal excretion of sodium leading to a fall in blood sodium levels. In chronic renal failure and other conditions such as cirrhosis of liver, there is retention of sodium and increased blood sodium and increased amounts of body fluids (eg: ascites, pleural effusion, edema). These are said to be due to an increase in aldosterone levels, probably due to its diminished destruction by liver. They may be also seen in Cushing's disease.

Losses of sodium also occur in excessive sweating (summer), diarrhoea and vomiting (through digestive secretions) and polyurea in diabetes, particularly if acidosis is also present.

Sodium is considered the backbone of body fluids, because the quantity of water in the extracellular fluid is regulated by the quantum of sodium in circulation.

Sodium and potassium on one hand and calcium, magenisum and the hydrogen ion on the other hand maintain the normal neuromuscular irritability.

$$\text{Irritability varies as:} \quad \frac{Na^{+} + K^{+}}{Ca^{++} + Mg^{++} + H^{+}}$$

Potassium: This is the principal cation of the intracellular fluid. About 4 grams are present in normal diets. Its deficiency is rare. Whole blood contains 200 mg/100 ml, but plasma contains only 20 mg/100 ml. (5 m.eq/litre). It is present in all tissues.

Together with sodium, it influences the neuromuscular activity of skeletal as well as cardiac muscle. A deficiency of potassium depresses the cardiac muscle. A gross excess also causes depression of cardiac muscle and paralysis of skeletal muscles.

Excretion is mainly through urine. Adrenal cortical hormones influence potassium metabolism in a direction opposite to that of sodium. Hyperfunction of the adrenal cortex (eg. Cushing's disease) causes increased loss of potassium in urine and decrease in plasma levels. Hypofunction of the adrenals (eg: Addison's disease) and renal failure cause an increase in plasma potassium levels.

Loss of potassium from tissues occurs in wasting diseases (potassium is lost along with tissue protein) and in severe dehydration. In severe diarrhoea and vomiting also potassium may be lost in large amounts. Certain diuretics (eg: acetazolamide or Diamox) also increase its urinary excretion. Tissue protein synthesis causes an uptake of potassium, about 2 mg per each gram of protein, and synthesis of glycogen in liver and muscle will take up about 3 to 4 mg of potassium for each gram of glycogen.

Chlorine: It exists mainly as sodium chloride in blood and plays a role in the water balance and osmotic pressure and pH regulation. It is also necessary for the formation of HCL by the gastric mucosa.

It is taken as sodium chloride mainly and a deficiency or surplus of sodium and chloride occur together. The chloride content of cerebrospinal fluid is higher than that of plasma on account of the lower protien content. Chloride balances the anion content of C.S.F. Its concentrations in different body fluids are as follows:–

Whole blood	..	250 mg/100 ml. or 70 m.eq/litre
Plasma	..	365 mg/100 ml. or 103 m.eq/litre. (as NaCl 600 mg/100 ml.)
C.S.F.	..	440 mg/100 ml. or 124 m.eq/litre. (as NaCl 720 mg/100 ml..)

Disturbances in Electrolyte Composition of Body Fluids:

Changes in sodium and potassium concentrations are of interest from the clinical point of view.

Sodium deficit: The extracellular fluid (ECF) sodium might be lost with or without simultaneous fluid loss. This can occur in severe burns, peritonitis, large ascites and pleural effusion and severe edema. The loss of sodium in these cases is also accompanied by loss of plasma albumin. Sodium loss can also occur in severe vomiting or diarrhoea. Administration of cation-exchange resins may also cause it. Excessive sweating may lead to substantial losses of sodium through the skin. Added to this, if lot of plain water is ingested to allay thirst, the resulting dilution of plasma magnifies the sodium deficit even more, and when the kidneys remove the extra water, there is a further loss of sodium through the urine.

Inability of the renal tubule to reabsorb normal amounts of sodium can occur in salt losing nephritis. Water retention due to excessive ADH action can also cause dilution of plasma and low sodium levels. In Addison's disease, the plasma sodium levels are low. In conditions like diabetic acidosis, therapy with mercurial diuretics or acetazolamide, there is an increased urinary excretion of sodium.

The affected subject will show weakness, apathy, headache, giddiness, muscular cramps and in severe depletion, delirium and coma may occur. The eyeballs are shrunken and soft, tongue is shrunken and wrinkled, and skin is inelastic.

Packed cell volume (hematocrit) is usually increased and serum sodium lowered. Serum potassium and urea are increased. Urine volume and particularly sodium content of urine are markedly decreased.

The condition is treated by administering isotonic saline or plasma intravenously. In a few cases, hypertonic saline (3– 5%) is indicated. Where the sodium deficit is due to loss of gastrointestinal secretions (vomiting, diarrhoea), there is likely to be a potassium deficit also. This has also to be made good.

Sodium Excess: Serum sodium concentration does not reflect the total body sodium levels. Low serum sodium level can be associated with low, normal or high body sodium. Similarly, a high serum sodium may also be associated with any of the above three states of body sodium.

Increase in total body sodium is always associated with edema. It can be due to circulatory failure, excessive production or excessive action of aldosterone and ADH.

The underlying cause of edema has to be treated.

Hypopotassemia (Hypokalemia): This can occur in conditions of dehydration and alkalosis due to any cause. It does not indicate that the body potassium content is low. The usual causes of hypokalemia are–

(i) Infusion of large quantities of potassium-free fluids in the treatment of dehydration.

(ii) Alkalosis due to any cause.

(iii) Loss of potassium through gastrointestinal secretions (diarrhoea and vomiting).

(iv) Loss through urine due to excessive bicarbonate administration or due to renal tubular failure (potassium-losing nephritis); or administration of mercurial diuretics and

(v) Cushing's Syndrome.

Familial periodic paralysis' is a condition where hypopotassemia occurs spontaneously.

The conditions are treated by oral or parenteral administration of KCL and potassium rich diets like orange juice, bananas etc.

Potassium excess (Hyperkalemia):

Like hypernatremia, this also does not necessarily mean an increase in total body potassium. It only indicates a rise in serum potassium. It can occur in:

(i) Excessive intake of potassium salts.

(ii) Crush syndrome due to liberation of intracellular potassium from the injured tissue.

(iii) Addison's disease, and

(iv) Large volume of blood transfusion.

Muscular weakness, paralysis, cardiac failure and typical changes in the electrocardiogram may be observed. Serum potassium levels increase to 6 m.eq./litre or more. Serum sodium is usually decreased.

Treatment is by low potassium diet with low protein (protein foods contain much of tissue potassium), and hypertonic glucose (25%) and insulin parenterally. Anabolic steroids are also administered. The latter two, by stimulating glycogenesis and protein synthesis, cause the passage of serum potassium into the cells (2mg. K^+ for each gram of protein synthesized and 3–4 mg.K^+ for each gram of glycogen synthesized). Cation exchange resins may be given by mouth to remove potassium from the gastrointestinal secretions and prevent its reabsorption from the gut.

CALCIUM

Quantitatively this is present in largest amount on account of its being the main mineral constituent of bone and teeth. Small amounts are present in blood and body fluids and exert a regulatory function (depressent effect) on neuromuscular irritability and blood coagulation and also in the permeability of cell membranes and capillaries.

It is required for regulating a large number of cellular activities, nerve and muscle function, hormonal action, blood coagulation and cell motility. These actions are mediated by an intracellular protein - *CALMODULIN* - which is present in all nucleated cells and acts as a receptor molecule for Ca^{++}. Phenothiazine drugs act as potent inhibitors of calmodulin action.

Unlike potassium, which is present in all sources of food, calcium is present only in milk and milk products, egg yolk, legumes, nuts and green leafy vegetables. Adult man requires about 400–500 mg. per day. The metabolism of this mineral is closely interrelated with phosphorus metabolism and is regulated by vitamin D and parathormone.

Absorption: Unlike sodium and potassium which ae readily absorbed from the intestines, the absorption of calcium is incomplete. A high protein diet favours its absorption whereas a high cereal diet will diminish it. On a high protein diet, 15% of dietary calcium is absorbed. If the protein content is low, only 5% might be absorbed. The cereals contain phytic acid (inositol hexaphosphate) which forms an insoluble salt with calcium. Oxalates, present in vegetables like cabbage and spinach, also prevent its absorption. An acidic pH of the intestine favours absorption. A ratio of food calcium to phosphorus not more than 2:1 and not less than 1:2 is necessary for optimal absorption of calcium. Vitamin D promotes its absorption. Presence of large amounts of unabsorbed fatty acids, as in sprue syndrome, will cause its excretion as calcium soap.

Two mechanisms are employed for the absorption of calcium by the intestinal mucosal cells.

1. An active transport process involving metabolic energy and a Ca^{++} pump.

2. Simple diffusion.

Both the processes require 1, 25-dihydroxycholecalciferol. It regulates the synthesis of the proteins involved in Ca^{++} binding and transport and also Ca^{++} -dependent ATPase.

Glucocorticoids inhibit Ca^{++} absorption by some, as yet unknown mechanism.

Plasma contains 9.0–11.0 mg/100 ml (5 m.eq/litre) of which about a half is in an ionizable and diffusible form and another half is protein-bound and non-diffusible. Small amounts also occur as citrate which is non-ionizable but diffusible. Erythrocytes do not contain any calcium. The C.S.F. contains about 4.0–5.0 mg/100 ml. (2 m.eq./litre) of calcium, mainly in the ionizable form.

The effect of calcium on neuromuscular excitability (depressor effect) and on coagulation of blood are on account of the ionizable fraction.

The calcium in the glomerular filtrate is almost completely reabsorbed by the tubule. Only small amounts are present in urine (about 200 mg. in 24 hours).

In the proximal convoluted tubule, calcium absorption is associated with sodium reabsorption. In the distal tubule calcium is actively transported unrelated to sodium reabsorption. Parathyroid hormone stimulates this active reabsorption by the distal convoluted tubule.

Excretion of calcium is mainly through feces and reflects the unabsorbed portion of dietary calcium.

Abnormalities in calcium metabolism: Parathormone causes alterations mainly in the diffusible portion of calcium in plasma.

Hyperparathyroidism: In this condition the plasma calcium levels will reach upto 12–20 mg/100 ml. There is a corresponding decrease in phosphate level. The increase of calcium in plasma is mainly by demineralization of bone leading to entry of calcium and phosphorus from bone into blood.

By diminishing tubular reabsorption of phosphate in the kidney, the parathormone causes an increase in its urinary loss and lowers serum phosphate levels.

There is also increased excretion of calcium in urine.

Hypoparathyroidism: In this condition, the serum calcium levels (particularly the ionized and diffusible fractions) are reduced to 7 mg/100 ml or below and the serum phosphate levels increase due to a decreased excretion in urine. A condition called 'tetany' occurs characterized by increased neuromuscular irritability, spasms and convulsions.

Rickets: Apart from a deficiency of vitamin D, a deficit in calcium and phosphorus in the diets of children can lead to the development of rickets. In children, serum inorganic phosphate levels vary from 4–7 mg per 100 ml. In rickets, it may fall to as low as 1–2 mg%.

Relationship to plasma phosphate: There is a sort of an inverse relationship with plasma phosphate levels. The plasma Ca × P product is $10 \times 4 = 40$ in a normal adult.

The Ca × P product in children is normally 50. It is lowered to 30 in rickets.

Renal rickets: Here the primary defect is in the absorption of calcium and phosphorus from the intestinal epithelium and the reabsorption of phosphate by the renal tubule. There is a gross increase in the urinary excretion of phosphate (hyperphosphaturia) and lowered blood calcium and phosphate levels. The condition is not relieved by vitamin D and is hence called 'vitamin-D resistant rickets'. It is a familial disease, transmitted as an X-linked dominant trait, affecting male children.

Relationship to plasma proteins: Since about 5 mg/100 ml of plasma calcium is protein-bound, chiefly to the albumin fraction, any decrease in plasma proteins (hypoproteinemia) is associated with a decrease in the non-diffusible, protein-bound fraction of calcium. The role of calcium in bone formation had been described under vitamin D.

PHOSPHORUS

Like calcium, it forms a predominant constituent of bone and teeth. But it is also present in every cell of the body in combination with proteins, lipids and carbohydrates (eg: phosphoproteins, phospholipids and hexose phosphates). As a constituent of ATP and similar compounds, it has a unique role in energy storage and transformations.

About 800 mg are required per day for an adult. In occurs together with calcium in most of the sources mentioned for calcium. Proteins of food which do not contain calcium also supply good amounts of phosphorus. Blood plasma contains 3–5 mg/100 ml. as inorganic phosphate (1.0–1.5 mM/litre). Total blood phosphorus is 40 mg/100 ml.

The absorption of phosphorus from the intestines takes place under conditions identical to those for calcium absorption. Excretion is mainly through urine (unlike calcium). Only small amounts are excreted in feces.

Plasma contains, besides inorganic phosphate, lipid phosphate and organic phosphate. The inorganic phosphate levels show a slight reduction during metabolism of carbohydrate (due to formation of hexose phosphates). The Phosphate level decreases in hyperparathyroidism and increases in hypoparathyroidism.

Parathormone decreases the reabsorption of phosphorous from the proximal, as well as distal convoluted tubules, and causes increased excretion of phosphorous in urine.

In renal failure, there is an increase of plasma phosphate levels due to a decrease in excretion. The reverse occurs in renal rickets. There is an increased excretion of phosphate in renal rickets as well as in Milkman's syndrome and deToni-Fanconi syndrome leading to a decrease in plasma phosphorus. In the last condition, there is glycosuria and an increased excretion of amino acids and phosphorus in urine due to decreased reabsorption of glucose, amino acids and phosphates by the tubule.

BONE

Bone consists of cells – osteoblasts and osteoclasts – and matrix in which characteristic mineral salts are deposited. Inside the bone cavity is present the marrow which is rich in lipids (25%), mostly triglycerides. The matrix contains three main groups of proteins:

(i) a collagen-like protein – ossein,

(ii) an elastin-like protein – osseoalbuminoid, and

(iii) a mucoprotein, rich in chondroitin sulfate – osseomucoid.

The minerals present are 1. cations: mainly calcium; small amounts of sodium, potassium and magnesium. 2. Anions: mainly phosphate, small amounts of carbonate, citrate, chloride and fluoride.

Bone formation: This consists of two distinct processes–

1. Formation of the organic matrix.
2. Mineralization of the matrix.

1. *Formation of the Matrix:* In the areas of developing bone, reticular cells of the bone marrow turn into osteoblasts and lay down the intracellular matrix or osteoid, consisting of parallel bundles of collagen fibres and a ground substance made up of mucoprotein and mucopolysaccharide. The osteoblasts are rich in alkaline phosphatase, an enzyme capable of liberating inorganic phosphate from its organic esters. The osteoblasts are also rich in glycolytic enzymes.

Vitamins C and A are required for the normal activity of osteoblasts in laying down the matrix. Growth hormone, androgens and thyroid in physiological amounts are also essential. Adrenal cortical hormones and thyroid hormones in excess, on the other hand, on account of their interference with the synthesis of chondroitin sulfate and increased protein catabolism, have an adverse effect on osteoid formation.

A small protein called '*osteocalcin*', with three gammacarboxylate residues, binds strongly to the hydroxyapatite crystals. It helps in regulating Ca^{++} in bone and teeth. Since vitamin K is essential for the formation of the γ -carboxyglutamate, the vitamin has a role in the metabolism of bone and teeth.

2. *Mineralization:* The mineral constituent of bone is mainly hydroxyapatite-$[3\,Ca_3(PO_4)_2]$ $Ca(OH)_2$.

The local factors that influence the formation and deposition of this salt from the available calcium and phosphate of plasma are not clearly understood. The presence of large concentration of alkaline phosphatase and glycogen, and the observation that glycogen rapidly disappears before or during the mineralization process, were interpreted to mean that the phosphatase enzyme acted on the phosphate esters of glucose and other phosphorylated products of glycolysis and liberated inorganic phosphate in quantities sufficient to raise the Ca×P product to cause their precipitation as hydroxyapatite. But this view is doubted now. The role of alkaline phosphatase might be only in the laying down of the matrix. Glycolysis probably provides the required energy for this and other processes. The collagen fibres themselves, embedded, as they are, in the highly polymerized mucopolysaccharide (of the ground substance) act as templates for the crystal formation. The ground substance behaves like a polyelectrolyte and causes precipitation of Ca^{++} and $HPO_4^{=}$ ions into the collagen fibres. Once the hydroxyapatite crystals are formed, crystal growth occurs spontaneously.

The crystal lattice formed offers a very large surface area – about 100 square meters per gram of bone tissue. This large area is in contact with the extracellular fluid and is in dynamic equilibrium with it. It acts like an ion-exchange surface and good amounts of sodium, carbonate, citrate and other anions as well as cations get incorporated into the bone crystals. Heavy metals like lead, uranium, radium and strontium, if present in circulation. get deposited in the bone crystals. During conditions of acidosis, some of these cations, particularly sodium, are drawn out of the bone. In alkalosis, the reverse occurs. The cations of plasma are deposited in the bone lattice. Even under normal conditions, there is a constant exchange of minerals between plasma and bone. The inorganic material comprises of about one fourth the volume of bone, but on account of its density, it accounts for one half of the weight of bone.

TOOTH

Tooth consists of three layers of calcified tissue-

(i) *dentine,* which surrounds the pulp cavity and extends throughout the entire portion of tooth,

(ii) *cementum,* a layer covering the portion of tooth lying buried in the gum, and

(iii) *enamel,* a white, hard material covering the portion of tooth projecting above the gum.

Dentine is hard and dense and consists of 75% mineral. Enamel is even more dense – it is the hardest substance present in the body and contains 98% mineral. The organic matrix of dentine and cementum is bone like. The enamel in the embryonic tooth is made up of a fibrous protein of high proline content. There is also appreciable amount of hydroxylysine.

In the fully formed enamel, much of protein disappears. Only few small peptides resembling keratin and devoid of proline remain. The hydroxyapatite crystals of enamel are quite large compared to that of bone. The turnover rate of phosphate in dentine is one sixth that of bone, while the turnover rate in enamel is even slower – one hundredth of that of bone. Hence, even in conditions of generalized decalcification of bone, teeth are little affected.

As in the case of bone, vitamins A, C and D are all necessary for the proper tooth development. Lack of vitamin A causes hypoplastic enamel, imperfectly calcified. Lack of vitamin C affects calcification of dentine. Vitamin D is required not only for the absorption of calcium from the gastrointestinal tract, but also for the proper deposition of calcium and phosphorus in tooth. Apart from calcium and phosphate, other ions like magnesium, chloride, carbonate and citrate are all present in tooth just as in bone.

Dental Caries: When the enamel breaks down and the dentine is exposed, caries can develop. Food particles lodged between the teeth undergo decay due to bacterial action and produce acids (eg: lactic acid). This dissolves enamel and dentine. Sweets, candies and pastries are more easily fermentable and are therefore the worst offenders in this regard. A saliva rich in mucin has less cleaning action on tooth and therefore predisposes to the development of caries. A saliva of low mucin content, on the other hand, protects against caries. In addition to the acid metabolites, bacteria also produce proteolytic enzymes which can act on the small amounts of the protein in the enamel and synergize the acid action.

Others believe that a basically poor architecture of the tooth is necessary for the development of caries. Poor nutrition of the infant and the mother at the time of early infancy and childhood result in laying down teeth of substandard quality which become easily suceptible to the development of caries in later life.

Fluroide in low concentrations (1–2 parts per million) if present in drinking water is found to have a beneficial effect in decreasing the incidence of caries in the population. It is probably an essential element in the composition of enamel and its presence might make the enamel resist the solvent action of acids; it may also act locally as an inhibitor of the enzymes that produce the organic acids.

MAGNESIUM

Seventy per cent of body magensium occurs in bone in association with calcium and phosphorus. The rest is present in all the cells of the other tissues and in blood and body fluids. Blood contains 2–4 mg/100 ml (1.7 to 3.4 m.eq/litre). It is about equally distributed between cells and plasma. C.S.F. also contains about 3 mg/100 ml.

It is necessary for the activity of several enzymes in carbohydrate metabolism (eg: hexokinase, phosphofructokinase, phosphoglyceratekinase, enolase, pyruvate kinase, adenyl cyclase, phosphoglucomutase, pyruvate dehydrogenase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, transketolase) and others; (in some of these, calcium or manganese may replace Mg^{++}). Together with calcium and hydrogen ions, it depresses neuro-muscular activity and balances the action of Na^{+} and K^{+}.

300–350 mg/day are required for adult. Nuts, beans, cereals, vegatables, meats and milk supply adequate amounts in a normal diet. High amounts of calcium and phosphorus in diets and ingestion of large amounts of alcohol will decrease magnesium absorption. Normally about 50% of dietary magnesium is absorbed.

Excretion is mainly through feces (unabsorbed dietary magnesium). Absorbed magnesium is excreted through urine, bile and intestinal secretions. Increase in plasma levels of magnesium depress the nervous system and if sufficiently high can induce anaesthesia and paralysis of skeletal muscle. Magnesium and potassium are normal cations of the intracellular fluid. Aldosterone causes in increased urinary excretion of both potassium and magnesium.

Low levels of serum magnesium cause symptoms similar to tetany.

In hyperparathyroidism, along with depletion of calcium there is also a depletion of magnesium from bone and both are excreted in large amounts in urine.

Renal failure may produce a rise in plasma magnesium levels and this may explain some of the findings like depression and muscular weakness in uremia.

SULFUR

This is present in all cells in association with protein in the sulfur containing amino acids, cysteine and methionine. It is ingested in the form of protein mainly and is eliminated in the urine in three forms described as inorganic, organic and etheral sulfates totalling about 0.5 to 1.0 gram a day. These are described in detail under metabolism of sulfur containing amino acids. The formation of inorganic sulfate occurs mainly in the liver.

It plays an important role in detoxication of phenolic compounds and steroidal hormones. For these conjugation reactions, the inorganic sulfate is activated by ATP to form adenosine-phospho-sulfate or 'active sulfate' (Adenine-ribose P-O-S-$O_3^=$).

Cystine, methionine, glutathione, heparin, insulin, anterior pituitary hormone, thiamine, lipoic acid, biotin, coenzyme A, ergothionine, and taurocholic acid are some of the sulfur containing compounds.

IRON

Iron, as a component of hemoglobin, myoglobin and the cytochromes, plays a key role in oxygen transport and cellular oxidations.

Adult man requires to take about 10 mg. iron daily in his diet. Growing children and pregnant and lactating women require more. Even non-pregnant women require more on account of the periodical loss of blood during menstruation. Organo-meats like liver, heart and kideny, egg yolk, green leafy vegetables, whole wheat and molasses are good sources.

Iron content of some tissues

Tissue	Total amount present	% of total body iron
Hemoglobin	2500 mg	67
Storage (ferritin and hemosiderin).	1000 mg	27
Myoglobin	130 mg	3,5
Other tissues	88 mg	2.4
Transport iron	3 mg	0.1

Absorption of iron: There is a self regulatory mechanism in the absorption of iron. In a normal adult in good health, less than 1 mg of iron is absorbed from dietary iron – just enough to replace an equivalent amount lost in urine. Thus only 10% of food iron is absorbed. During conditions of growth or during recovery following hemorrhage or iron-deficiency anemia, the rate of absorption is very much increased. The unabsorbed iron is excreted through feces.

Absorption occurs mainly from stomach and duodenum. The gastric HCL and other organic acids of food help in converting the iron in organic compounds in food to inorganic ferric iron. Ascorbic acid, cysteine and other reducing substances of food convert the ferric iron to ferrous form. It is the ferrous iron that is readily absorbed by stomach and duodenum. Operations wherein stomach or duodenum are removed (gastrectomy) will interfere seriously with absorption of iron due to deficiency of HCL and also the absorbing surface.

High phosphate diet also decreases iron absorption as the iron phosphate formed is not soluble. Similarly phytic acid and oxalates diminish iron absorption.

Absorption of iron:

Heme is absorbed unchanged by the intestinal mucosa and the iron is released from heme in the mucosal cell. Iron contained in other foods is absorbed in the ferrous state by the mucosal cells of the duodenum and proximal jejunum. It is immediately oxidized to ferric iron and is bound by an intracellular carrier molecule. From this carrier molecule, a part of the iron is transferred to apotransferrin (a protein with a molecular weight of 90,000). Each molecule of apotransferrin can take up two atoms of iron to form *'transferrin'*. This is the transport form of iron and enters the circulation. On electrophoresis, it migrates with the beta globulin fraction. Normally, only 20–33% of the iron binding capacity of transferrin is saturated.

The rest of the iron is taken up by *'apoferritin'* (a protein with molecular weight of 500,000). Each molecule can take up 4,300 atoms of iron. It is the storage form of iron and is called *'ferritin'*.

In case of iron deficiency, the capacity of the carrier molecule to take up iron is increased and more iron is passed on to form transferrin and less to form ferritin. Even the iron already stored in ferritin can be transferred to transferrin. Fe^{+++} has to be converted to Fe^{++} for the transfer to occur. It is reoxidized to Fe^{+++} in the transferrin molecule.

In iron overload, the reverse set of events occur. Excessive iron stored in the mucosal cells of the intestine as ferritin can be lost by shedding of the mucosal cells. The hormone *'erythropoietin'* promotes rapid transfer of mucosal iron to transferrin.

Transferrin transports iron through circulation to storage sites in bone marrow and liver where the iron is again incorporated into apoferritin of those tissues to form ferritin. When the iron load is excessive, the ferritin gets denatured and breaks into subunits which aggregate to form microscopically visible miscelles called *'hemosiderin'*. The iron content of hemosiderin is much more than that of ferritin. A deficiency of vitamin C favours the conversion of ferritin to hemosiderin.

For the formation of ferritin from apoferritin, Fe^{++} has to be converted to Fe^{+++}. This oxidation is brought about by apoferritin itself, which acts as a ferroxidase. Fe^{+++} is tightly bound to ferritin. For the release of iron from ferritin, it has to be reduced to Fe^{++}.

Transport of iron: For iron to enter or leave a cell, it has to be converted to ferrous form. It is immediately reoxidized to ferric form and combines with a specific metalbinding-protein called siderophyllin or transferrin which migrates with the β_1 -globulin in electrophoresis. It is a glycoprotein. Normally there is enough of this transferrin to bind about 360 μg of iron/100 ml blood. This is known as the 'total iron binding capacity' of plasma (TIBC). But plasma iron is only 90–120 μg/100 ml in females and 120–140 μg/100 ml in males. Thus only one third of TIBC is used, the rest being only a reserve provision.

There is a circadian variation in plasma iron levels which may be as much as 60 micrograms per 100 ml in a 24 hour period. The lowest values occur 2 hours after going to bed in the night and the highest values 7 to 9 hours after going to bed.

There is very little excretion of iron in urine because it is in combined state with protein. In proteinuria there is a significant excretion of iron in the urine.

Storage of iron: The liver, spleen and bone marrow all contain apoferritin which can store iron by being converted to ferritin. The passage of iron from transferrin to apoferritin follows similar steps as earlier. The iron is released as ferric iron from transferrin, reduced to ferrous form, taken up by the cells of the concerned tissues, reoxidized to ferric form and incorporated into apoferritin to form ferritin. If the iron supply is far in excess of demand (usually due to a breakdown in the mucosal block or due to parenteral administration), iron is stored in some of these viscera in a different form as 'hemosiderin' which is also a protein containing 35% iron. Hemosiderin is visible under the microscope with suitable staining, whereas ferritin is not.

Utilization of iron: The daily synthesis of hemoglobin requires about 27 mg a day. That much is liberated every day from the breakdown of hemoglobin and is almost completely reutilized. Thus very small amounts only are required from absorbed iron, unless there is a loss of blood as in hemorrhage. Iron is also required for the activity of certain enzymes eg: aconitase,

Deficiency of iron produces a hypochromic microcytic type of anemia. It occurs due to lack of iron in diet or a deficiency in its absorption due to absence of gastric HCL, extensive resections of gastrointestinal tract, sprue and steatorrhoea.

About 300 mg of iron is transferred to the fetus during intrauterine life through placenta. Since there is no loss by excretion, this amount will be sufficient for some months to the infant. Milk which is the main nutrient during this period, is poor in iron content.

An excess of iron rarely occurs as long as the mucosal block functions normally. But if this does not function well due to any reason (usually an inherited defect) or when iron is administered in large doses parenterally, hemosiderin accumulates in the skin, pancreas, liver, spleen and other sites leading to a bronzed appearance of skin, diabetes mellitus and cirrhosis of liver. The condition is called hemochromatosis or hemosiderosis.

A deficiency of vitamin C in diet causes deposition of iron as hemosiderin instead of as ferritin. Iron from hemosiderin is less readily available for heme synthesis. Hence an iron deficiency anemia occurs.

Bantu siderosis: Tribals of Africa called 'Bantus' ingest a high cereal, low phosphorus diet cooked in iron pots. This facilitates absorption of large amounts of iron and leads to a typical hemosiderosis.

COPPER

It is necessary for the activity of cytochromes, catalase, tyrosinase, monoamine oxidase and ascorbic acid oxidase. Traces of copper are required for normal synthesis of hemoglobin. The erythrocytes contain a colorless copper containing protein called erythrocuprein in small amounts. Cerebrocuprein is a copper containing protein present in the brain. Erythrocuprein, cerebrocuprein and another copper protein hepatocuprein in liver are all now identified to be an enzyme – cytosolic superoxide dismutase. It is a protein of 32,000 molecular weight and contains two cupric ions and two zinc ions per moleucle. Ceruloplasmin is a copper containing protein (bluish colored) present in plasma. It is considered to be identical with the enzyme polyphenol oxidase. It also helps in the conversion of ferrous iron of plasma to ferric iron and its incorporation into ferritin.

Serum contains copper bound to ceruloplasmin associated with the alfaglobulin fraction and also copper loosely bound to albumin fraction.

In the intestinal mucosal cell, copper is associated with a low molecular weight metal-binding protein called "metallothionein." Ceruloplasmin is a copper containing glycoprotein synthesized in the liver. It is not a transport form of copper.

Copper is excreted in the bile by the liver. Normally there is very little copper in urine.

A copper deficiency produced in experimental animals caused a fatal hypochromic microcytic anemia. Copper favours absorption of iron from intestines and also its transport from and to tissues. Deficiency causes abnormalities also in bone formation and graying of hair in experimental animals.

Menke's disease: Copper is taken up by the intestinal mucosal cells, but its passage into systemic circulation through the serosal aspect of the mucosal cell is blocked. Mental retardation, instability of body temperature, abnormal bone formation, susceptibility to infections and kinky, steely hair are some of the symptoms of this condition.

Wilson's disease (hepatolenticular degeneration:) Copper accumulates in liver and lenticulate nuclei of brain. Plasma copper levels and ceruloplasmin levels decrease. There is generalized amino aciduria. The causation apppears to be an increased absorption of copper from intestines and a diminished ability to form ceruloplasmin. Copper is thus mostly in the free form in plasma and readily deposited in tissues and also excreted by kidneys; damaging the kidneys in the process.

The daily requirement of copper is about 2.5 mg. This is present normally in most of the diets.

Copper toxicity: Poisoning by copper salts causes diarrhoea with bluish green stools. Saliva also shows a bluish green color. Acute hemolysis and renal impairment can occur.

IODINE METABOLISM

Iodine is mainly required for the synthesis of the thyroid hormones. A minimum of 25 microgram is required daily. The National Research Council (U.S.A.) has recommended a liberal intake of 100–200 microgram. Sea water has a high iodine content. Hence, vegetables and fruits and other foods obtained near the sea shore contain sufficient amounts of iodine to meet its requirements. But in places located too far inland or at very high altitudes, the iodine content of natural foods is low and has to be supplemented by adding small amounts of iodide to table salt.

Absorption and excretion: Food iodine is first converted to iodide and then absorbed from the intestines. Iodides can be also absorbed from lungs, other mucus membranes and even through the skin.

Excretion is mainly through the kideny (40–80%). Small amounts are also excreted through bile, saliva, skin, lungs and intestines. Milk of lactating women also contains iodide.

Blood iodine: In the resting state, serum contains 4–10 microgram of iodide per 100 ml. Most of this is bound to protein (protein-bound iodine, PBI) and represents the iodine contained in the circulating thyroid hormone. The PBI levels increase in hyperthyroidism and decrease in hypothyroidism. Oral intake of iodides causes a rise of serum inorganic iodine. This is not protein-bound.

Metabolism: There is a total of 50 mg. of iodine in the human body. Muscles contain about 50% of this. The thyroid contains as much as 20% of the body iodine. Skin and skeleton contain small amounts. The concentration in the thyroid is, however, the highest: 10–40 mg/100 gm. Thyroid iodine is mainly organic and is combined with the protein of the colloid as 'iodo-thyroglobulin'. Various stages of the hormone synthesis – diiodotyrosine, triiodothyronine and thyroxine – are all present in the protein.

The hormones are released as required, enter the systemic circulation, and are mostly degraded in the liver and excreted through the bile. Bile thus contains iodides and also some of the organic products of degradation of the thyroid hormones. In tissues, thyroxine is mainly converted to triiodothyronine and thyroacetate.

MANGANESE

A deficiency of this mineral produces slipped tendon disease (perosis) in the chicken. No symptoms are known to occur in humans from its deficiency. Some of the enzymes eg: phosphatases, arginase, carboxylase, isocitric dehydrogenase and cholinesterase require Mn^{++} for their activity.

COBALT

It is a constituent of vitamin B_{12}. In some of the lower animals like the rat, cobalt itself stimulates erythropoiesis. It is also said to liberate the hormone 'erythropoietin' which stimulates erythropoiesis. In ruminants the micro-organisms inhabiting the gastrointestinal tract synthesize B_{12} from the rumen, but only if cobalt is present in the pastures.

ZINC

It is an element essential for normal growth, reproduction and longevity of animals. It is a component of several enzymes–alcohol dehydrogenase, alkaline phosphatase, carbonic anhydrase, procarboxypeptidase and retinal – retinene reductase. It also forms a complex with insulin and helps in its storage and release from the beta cells. Zinc is necessary for maintaining the plasma concentration of vitamin A.

The zinc content of human leukocytes decreases to a tenth of the normal level in leukemia. Leukocytes contain a cytosolic superoxide dismutase, an enzyme which destroys the highly toxic superoxide ion (O_2^-) produced during aerobic metabolism. The enzyme has probably a bactericidal function in the leukocytes.

Human adult body contains about 1 to 2 grams of zinc. A fifth of it is in the skin. Bones and teeth, spermatozoa, prostate and epididymis also contain large amounts.

An average mixed diet contains 10–15 mg of zinc. This is more than adequate. Meat, liver, eggs, fish, milk and cereals are good sources. It is readily absorbed from the intestines. Excretion is mainly through feces. Even parenterally administered zinc is excreted through feces. Pancreas, liver, kidney and spleen show a high turnover rate of zinc. Pancreatic juice is rich in zinc.

Zinc deficiency: A deficiency of zinc in the soil can lead to its deficiency in the usual food sources mentioned above and can result in a deficiency in the human. Delayed wound healing and impairment of acuity of taste are earliest symptoms. A deficiency can also occur in alcoholic cirrhosis. The cirrhotic liver contains less zinc than normal livers. The role of zinc in alcoholic cirrhosis of liver requires further investigation.

FLUORINE

Traces of fluorine are absolutely necessary for the normal development of teeth and bones. It particularly has a protective action against the development of dental caries, particularly in infancy and childhood. It is also necessary for the prevention of development of osteoporosis in adults, particularly in post-menopausal women.

Normal bones and teeth contain minimal amounts of fluoride. Fluoride is present in drinking water in varying concentrations depending on the soil. It is rapidly absorbed from the intestines just like chloride. It remains in the E.C.F. and is taken up by bones and teeth. Excretion is mainly through urine. Concentration of 1 ppm (part per million) of fluoride in drinking water is optimal. This will supply 1 to 2 mg of fluoride daily. The incidence of dental caries in children in such populations is 60–70% less than in populations in areas where the water contains less fluoride.

Fluorosis: Intake of excessive amount of fluoride for long periods will result in a clinical condition called fluorosis. An early sign is mottling and discoloration of the enamel of teeth The bones increase in density, calcification occurs at the points of insertion of tendons into bones and bony exostosis develop. The water in such places contains as much as 10 to 45 ppm. of fluoride.

Fluoride in large doses can also cause acute poisoning by inhibiting several magnesium-activated enzymes. Fluoroacetate is an inhibitor of the citric acid cycle.

MOLYBDENUM

Traces are required for activity of xanthine oxidase and liver aldehyde oxidase.

SELENIUM

Its requirement for the human is not yet proved. But it is essential for many animal species. A deficiency of selenium produces hepatic necrosis, muscular dystrophy, necrosis of the cardiac muscle, and several other disorders in various experimental animals.

Selenium is a component of the enzyme which converts the reduced glutathione to its oxidized form. In the conversion, H_2O_2 is used up and its accumulation is prevented. It is also required in some of the immune mechanisms, the biosynthesis of ubiquinone and the biosynthesis of ATP in mitochondria. The renal cortex, pancreas, pituitary and liver contain high amounts of selenium.

Vitamin E has a beneficial role in some of the selenium deficiency conditions and vice versa. Vitamin E prevents the formation of peroxides from polyunsaturated fatty acids.

References

Bothwell, T.H. and Finch, C.A.: Iron Metabolism, Little Brown, Boston, 1962.

Bourne, G.H.: The Biochemistry and Physiology of Bone, Acad. Pr., N.Y., 1956.

Bowen, H.J.A.: Trace Elements in Biochemistry, Acad., Pr., N.Y., 1966.

Comar, C.L. and Brown, F. (eds): Mineral Metabolism., Acad., Pr., N.Y., 1960-64.

Goldberger, E.A.: Primer of Water, Electrolytes and Acid-Base Syndromes, 4th ed., Lea and Febiger, 1970.

Gopalan, C. and Narasinga Rao, B.S.: Dietary Allowances for Indians, I.C.M.R. Special Report Series, No. 60, 1971.

Irwing, J.T.: Calcium Metabolism, Wiley, N.Y., 1957.

Josephson, B.: Chemistry and Therapy of Electrolyte Disorders, Thomas, Springfield (ILL), 1961.

Maxwell, M.H. and Kleeman, C.R. (eds): Clinical Disorders of Fluid and Electrolyte Metabolism, McGraw Hill, N.Y., 1962.

National Academy of Sciences: Recommended Dietary Allowances, Natl. Res. Council Pub. 1694, Washington, 1968

Peisachi J., Aisen, P. and Blumberg, W. (eds): The Biochemistry of Copper, Acad. Pr., N.Y., 1966.

Underwood, E.J.: Trace Elements in Human and Animal Nutrition, Acad. Pr., N.Y., 1962.

Wolstenholme, G.E.W. and O'Connor, C.M. (eds): Bone Structure and Metabolism, Little-Brown, Boston, 1965.

Weisberg, H.F.: Water, Electrolyte and Acid-Base Balance, 2nd ed., Williams and Wilkins, Baltimore, 1962.

23

REGULATION OF pH OF BLOOD AND BODY FLUIDS

THE pH of extracellular fluid is normally 7.4. It alters within a limited range of 7.36 to 7.44. The pH of interstitial fluid may be slightly lower. The intracellular fluid has varying pH depending on the type of cell. In osteoblasts it may be distinctly alkaline (pH 8.0) and in cells of the prostate gland it may be acidic (pH 5.0). The erythrocyte fluid has a pH of 7.25. In most other tissues it is 7.0. The pH of the body fluids and the different tissues is maintained fairly constant and in equilibrium with one another.

Factors which tend to alter the pH of body fluids:

The metabolic processes of the body form mostly acidic substances and the regulatory mechanisms are mainly geared to deal with the acids produced, though they are also capable of dealing with bases.

Oxidation of carbon of food produces carbondioxide. In a normal adult this amounts to about 400 litres a day. Since it forms carbonic acid with water, it is equivalent to 36 litres of 1.0 N acid. The sulfur and phosphorus of proteins and lipoproteins form sulfuric acid and phosphoric acid. Other products such as pyruvic and lactic acids are also formed during metabolism.

Few food materials produce bases. Citrate salts of fruit juices may produce bicarbonate salts. The secretion of HCL by gastric mucosa and the bicarbonate-rich secretion of bile and pancreatic juice will also tend to induce changes in the pH.

Substances administered as drugs also behave similarly. If NH_4 CL is administered, the ammonia part forms urea which is neutral, leaving HCL behind. Sodium bicarbonate will lead to an increase of this base.

Regulatory mechanisms: There are mainly three mechanisms.

1. **Buffers of body fluids.**
2. **Respiratory mechanism.**
3. **Renal regulation.**

1. Buffer systems present in the body fluids: The first impact of an increase in acid or base is taken up by the buffers present in the body fluids. Plasma contains the following buffer systems:

$BHCO_3/H_2CO_3$, B_2HPO_4/BH_2PO_4, B. organic acid/organic acid, and B. protein/protein.

B indicates the bases Na^+, K^+, Ca^{++} or Mg^{++}. Quantitatively, in the extracellular fluids, Na^+ is the most important.

Of all these systems, the $BHCO_3/H_2CO_3$ is the most important since this is most readily adjustable by altering the rate of CO_2 removal by respiration as needed. Hence all other buffers will ultimately pass on the increase in acid (H^+) or base (OH^-) to this system.

1. $BHCO_3 + \underset{\text{acid}}{HA} \longrightarrow BA + H_2CO_3$

$$H_2CO_3 \longrightarrow H_2O + CO_2$$

2. $BOH + H_2CO_3 \longrightarrow BHCO_3 + H_2O$

Hence accumulation of acid metabolites tends to lower the $BHCO_3/H_2CO_3$ ratio by a transient increase of H_2CO_3 while accumulation of bases tends to raise the ratio by an increase of $BHCO_3$.

According to the Henderson-Hasselbalch equation for buffer systems:-

$$pH = pK_a + \log \frac{(BHCO_3)}{(H_2CO_3)}$$

where pH is that of the fluid containing the buffer, pKa the dissociation constant of the weak acid and $(BHCO_3)$ and (H_2CO_3) are the molar concentrations of the base and acid. Since the pH is 7.4 under normal conditions and since pKa for carbonic acid is 6.1,

$$\therefore 7.4 = 6.1 + \log \frac{(BHCO_3)}{(H_2CO_3)}$$

$$\therefore \log \frac{(BHCO_3)}{(H_2CO_3)} = 1.3$$

Taking the antilog of 1.3

$$\therefore \frac{(BHCO_3)}{(H_2CO_3)} = 20$$

Under physiological conditions with a plasma pH of 7.4, the ratio of bicarbonate to carbonic acid is therefore 20.

Similar buffers are present in all body fluids. In the case of lymph and interstitial fluids, the protein content is less. In the erythrocyte, the protein is hemoglobin and the bicarbonate is mostly the potassium salt. In the case of other cells, the intracellular buffer is mainly protein, which is highly concentrated, and next in importance come the phosphate salts of potassium and magnesium.

When there is an increase in the acid content of the plasma, this is finally reflected as an increase in H_2CO_3 content. Acidity and CO_2 increases are both powerful stimulants of respiratory mechanisms and cause an increase in the rate and depth of respiration. The H_2CO_3 is converted to H_2O and CO_2 and the CO_2 is rapidly breathed out.

An increase in base, on the other hand, leads to a decrease in acidity and H_2CO_3 content (relative to $BHCO_3$). Respiratory rate and depth are both depressed, resulting in retention of CO_2 and thus an increase of H_2CO_3 till normal ratio of $BHCO_3/H_2CO_3$ is reached.

Renal mechanism: The pH of the urine is highly variable between 4.8 to 8.0. Normally the pH is slightly on the acid side. But this varies with the nature of diet, exercise etc. While the volatile acid H_2CO_3 is removed mainly by respiratory mechanism, the fixed acids like phosphoric, sulfuric and hydrochloric have to be removed by the kidney. A 24 hour urine of a normal individual contains the equivalent of 20 to 40 ml of 1.0 N acid and this is referred to as the titrable acidity of urine. The most important buffer of urine is B_2HPO_4/BH_2PO_4. At the pH of 7.4 this ratio is 5. When there is accumulation of acid (H^+) in the plasma, this is removed by the kidney by increasing the amount of BH_2PO_4 so that at a urinary pH of 4.8, the ratio may become 1/99. At an alkaline pH of the urine, say 8.0, the B_2HPO_4 is very much increased so that the ratio becomes 95/1. The detailed mechanisms by which kidney can function with such wide latitude depending upon the demand to remove H^+ion or the base is dealt with in the next chapter.

Disturbances in acid-base balance: Alterations in pH of blood may be due to a primary defect in elimination of CO_2 or due to metabolic causes and are classified accordingly.

Respiratory acidiosis: The H_2CO_3 content of plasma is increased. This occurs in conditions such as pneumonia, emphysema, congestive heart failure and poisoning with opium or barbiturates which depress the respiratory centre, thereby interfering with the CO_2 elimination by the lung. This is to some extent compensated by an increased resorption of HCO_3^- by the kidney to maintain the ratio $BHCO_3/H_2CO_3$ at 20.

Respiratory alkalosis: There is a fall in the H_2CO_3 level of plasma. This is due to hyperventilation in the lungs. It can occur in hysterical hyperpnoea and at high altitudes. This is compensated to some extent by an increase in the bicarbonate excreted by the kidney.

Metabolic acidosis: The bicarbonate fraction of plasma is decreased. This can occur in diabetes mellitus (ketosis and acidosis due to excessive loss of the fixed base Na^+ in combination with keto acid), renal failure (due to inability to exchange H^+ and NH_4^+ with Na^+) and severe diarrhoea and vomiting (due to loss of the bases of the gastrointestinal secretions).

Compensation in initial stages occurs by increased respiration whereby more CO_2 is eliminated to maintain the ratio of $BHCO_3/H_2CO_3$.

Metabolic alkalosis: There is an increase in the bicarbonate of plasma. Ingestion of alkalies in large amounts in treatment of peptic ulcer and vomiting due to high intestinal obstruction (which causes only the acidic gastric juice to be lost) are the two common causes. There is also a loss of chloride. Compensation is attempted by a depression of respiration so that more CO_2 is retained and by an excretion of an alkaline urine by the kidneys.

Assessment of the acid-base balance: Since $BHCO_3/H_2CO_3$ is the all important factor in determining the pH of the blood, and since an increase or decrease of the one is compensated by a corresponding increase or decrease of the other before failure sets in, any disturbance in the pH regulation of blood is always associated with changes in the total CO_2 (derived from $BHCO_3$ as well as H_2CO_3) of plasma.

CO_2 capacity or CO_2 combining power of blood: The forearm is immersed in warm water for a few minutes and blood is drawn from the antecubital vein and collected in a narrow mouthed glass bottle after addition of anticoagulant. The sample is equilibrated with normal alveolar air by blowing air into the bottle and rotating the bottle so as to form a thin layer of the blood on the sides of the bottle. The blood now contains H_2CO_3 and $BHCO_3$ at pCO_2 of 40 mm Hg. A measured sample of the plasma or whole blood from above is treated in a suitable gasometer (such as the Van-Slyke's) with acid to evolve CO_2 from H_2CO_3 as well as from $BHCO_3$. A vacuum is created in the apparatus to drive the CO_2 out of the solution and the volume of CO_2 is measured. The result is expressed as so many volumes of CO_2 per 100 volumes of blood. It is normally 55 to 75 volumes per cent. It is decreased in metabolic acidosis and increased in metabolic alkalosis (due to decrease and increase of HCO_3^- mainly).

In respiratory acidosis it is increased, and in respiratory alkalosis it is decreased, if the condition is compensated by alteration in HCO_3. In uncompensated cases, since the change involves only H_2CO_3 which forms 1/20 of the total CO_2, there is little perceptible change in total CO_2.

Alkali reserve: Since the bicarbonate of plasma is the fraction which shows alterations in metabolic conditions affecting the pH and is used to absorb the effects of increased acid metabolites, the bicarbonate content of plasma is referred to as the alkali reserve of the plasma.

Assuming a HCO_3^-/CO_2 ratio 20/1, the bicarbonate content can be calculated from the CO_2 capacity in vols% by dividing it with 2.24. This gives the bicarbonate in m.eq./litre. Normal bicarbonate content is 25–30 m.eq./litre.

Anion gap: This is a measure of the acid-base disturbance and is particularly useful in evaluating metabolic acidosis. The difference between the concentration of the main cation Na^+ and that of the main anions CL^- and HCO_3^- is referred to as the 'anion gap' [$Na^+ - (Cl^- + HCO_3^-)$]. Normally this is 142 – (103 + 27) = 12 m.eq/litre. This is increased in metabolic acidosis — say diabetic ketoacidosis — due to loss of HCO_3^-. In acidosis due to severe diarrhoea with loss of pancreatic and intestinal secretions, there is concomitant rise in Cl^- which compensates loss of HCO_3^-. The anion gap hence remains normal. The condition is called '*hyperchloremic metabolic acidosis*'.

The salient features for evaluation of acid-base balance are listed in Table 23-1.

TABLE 23-1

Evaluation of Acid-Base Balance

Type of disturbance	*pH*	*pCO_2*	*HCO_3^-*	*CL^-*
Respiratory acidosis	decreased	increased	increased	decreased
Respiratory alkalosis	increased	decreased	decreased	increased
Metabolic acidosis	decreased	decreased	decreased	variable
Metabolic alkalosis	increased	increased	increased	decreased

References:

Christenson, H.N.: Body Fluids and the Acid-Base Balance, Saunders, Philadelphia, 1964.

Davenport H.W.: The A B C of Acid-Base Chemistry, 4th edn., Univ., Chicago Pr., 1958.

Frisell, W.R.: Acid-Base Chemistry in Medicine, Macmillan, N.Y., 1968.

Goldberger, E: A Primer of Water, Electrolyte and Acid-Base Syndromes, Lea & Febiger, Philadelphia, 1962.

Muntwyler, E.: Water and Electrolyte Metabolism and Acid-Base Balance, Mosby, St. Louis, 1968.

Siggard, Anderson, O.: Acid-Base Status of Blood, 2nd ed., Williams & Wilkins, Baltimore, 1964.

Welt, L.G.: Clinical Disorders of Hydration and Acid-Base Equilibrium, Little-Brown, Boston, 1955.

Weisberg, H.F.: Water, Electrolytes and Acid-Base Balance, 2nd ed., Williams & Wilkins, Baltimore, 1962.

24

RENAL FUNCTION: FORMATION AND COMPOSITION OF URINE

THE kidney not only excretes the nonvolatile metabolic waste materials, but is also intimately concerned in the maintenance of the homeostasis of the body fluids. Kidney function can be considered to consist of four phases.

1. Filtration of protein-free plasma by glomeruli.

2. Selective reabsorption by the tubule.

3. Secretion by the tubule.

4. Maintenance of acid-base balance.

The nephron is the functional unit of the kidney. A tuft of arteriolar capillaries forms a glomerulus. This is surrounded by a double-walled epithelial structure called Bowman's capsule in the form of a funnel. This leads on to the tubule which consists of a proximal convolution, a descending limb, loop and an ascending limb of the loop of Henle, leading into the distal convolution. This leads on into a collecting tubule which finally empties into the pelvis of the ureter.

Formation of the glomerular filtrate:

During rest, 25% of cardiac output *i.e.*, about 1 litre of blood passes through both kidneys. Direct measurements show that the hydrostatic pressure in the glomerular capillary averages 45 mm Hg i.e., 40% of mean aortic pressure. The oncotic pressure due to plasma proteins is about 20 mm Hg. at the beginning of the capillary. This and the hydrostatic pressure in the tubule of 10 mm Hg. oppose filtration. There is thus a net effective filtration pressure of 15 mm Hg. (45 minus 20 minus 10) with which the glomerular filtrate can be formed. The filtrate is essentially a protein and cell-free plasma. There are about 2 million nephrons in the two kidneys together and they form about 120 ml of filtrate per minute from the 1 litre of blood circulating through them. The glomerular filtration rate (GFR) is thus 120 ml minute. The filtrate contains all other constituents of plasma except the proteins.

Function of the tubule: Though the filtrate is identical with plasma in all solutes except proteins, the urine that is finally excreted has altogether a different composition. Some substances which are necessary for the body are reabsorbed along with nearly 99% of the water of the filtrate. Certain other substances are added to produce the final urine which is passed out into the bladder at the rate of about 1 ml per minute only.

Selective reabsorption by the tubule:

Certain substances like glucose and amino acids are completely absorbed from the tubule when they are present in physiological concentrations in the filtrate. They are excreted only when their concentrations exceed a particular limit known as the threshold value for that substance. Glucose and amino acids are thus called 'high-threshold substances.' In the case of glucose, the tubule has the capacity to absorb about 350 mg per minute. This is known as the tubular maximum for glucose absorption (Tmg). With normal blood sugar levels ranging from 70 to 130 or 150 mg/100 ml this amount is rarely exceeded in the filtrate. If however the sugar rises to over 170 or 180 mg%, then the filtrate formed per minute contains over 350 mg and some glucose is excreted in urine. The blood sugar level beyond which glucose makes its appearance in urine is known as the 'renal threshold for glucose.'

Low-threshold or no-threshold substances: Others like creatinine, urea and uric acid are always excreted in varying amounts independent of their concentration in plasma. These are called 'no-threshold' or 'low-threshold' substances. The tubule is able to regulate the absorption of these several substances by as yet poorly understood selective absorptive mechanisms.

Water reabsorption: The solutes mentioned above are all reabsorbed along with adequate amounts of water in the form of aqueous solution. In the proximal tubule the solutes are absorbed mainly as isotonic solution and the reabsorption of water is thus secondary to the reabsorption of solute. If more solute is to be absorbed, more water is reabsorbed and vice versa. This process mainly occurs in the proximal convoluted tubule and the descending limb of the loop of Henle and is known as 'passive' or 'obligatory' reabsorption. This accounts for about 80% of the water reabsorbed.

The filtrate reaching the loop of Henle is thus only about 20% of the original volume and is still isotonic with blood plasma.

According to Wirz, the filtrate becomes concentrated as it passes down the descending limb of the loop which is embedded in the renal medulla and papillae which themselves are hyperosmotic with respect to plasma. Hence water is lost from the descending limb to make the tubular fluid also hyperosomotic. This loss of water in the descending limb is unaccompanied by solute. In the ascending limb, the reverse set of actions occur. The tubule is passing from a hyperosmotic zone to a hyposmotic zone. To equilibrate with the surrounding tissue, there is active secretion of NaCL unaccompanied by water in the ascending limb, so that the tubular fluid becomes hyposmotic with plasma. This is known as the 'counter-current theory' of Wirz.

Role of urea in countercurrent distribution:

Urea is not secreted in the Henle's loop and hence gets concentrated in the fluid reaching the distal convoluted tubule which is readily permeable to urea. Urea diffuses out into the interstitial tissue and increases the tonicity of that tissue. Some urea may be reabsorbed into the ascending limb of Henle's loop.

The distal convoluted tubule and the collecting tubule are relatively impermeable to water. But they become permeable under the influence of the antidiuretic hormone (ADH). The remaining 19% or so of the filtrate is thus absorbed in this portion of the tubule under hormonal influence. This is independent of any necessity to reabsorb solutes. Water is absorbed for its own sake and possibly involves an active process. This phase of water reabsorption is called the 'facultative reabsorption.'

The stimulus for liberation of ADH is through osmoreceptors located in the anterior hypothalamus. The regulation of body water by this mechanism was already dealt with. Alcohol supresses ADH secretion and causes diuresis. Conditions of stress such as trauma or surgery cause excessive production of ADH and lead to diminished urine output and retention of water. The ADH binds to a specific receptor in the basal plasma membrane of the collecting tubular cell. This initiates the process of formation of cyclic AMP in the cell. The cyclic AMP; in turn, stimulates protein kinases and some of the proteins of the membrane on the lumen side of the cell become phosphorylated. This makes the membrane more permeable to water.

Tubular secretion:

Certain substances are also actively secreted by the tubule in addition to the filtration by the glomerulus. Uric acid and creatinine (when its blood levels are high or when exogenous creatinine is administered) are secreted by the tubule. A number of foreign substances like phenol red, diodrast and iodohippurate are also actively secreted by the tubule. The maximum rate at which a substance can be secreted by the tubule is measured as the Tm secretory rate. For para-aminohippurate, this is about 80 mg/minute.

Role of tubule in regulating plasma pH:

Sodium, the fixed base of the body, and chloride are absorbed selectively by the proximal tubule in isotonic solution. Potassium in physiological amounts is also completely reabsorbed by the proximal tubule. Some amount of potassium is added later by secretion by the distal tubule in exchange for sodium. The adrenocortical hormones favour the reabsorption of sodium and excretion of potassium and thus regulate the mineral levels in the blood and body fluids. This aspect was already reviewed under mineral metabolism.

Nonvolatile acids such as lactic acid, ketone bodies, sulfuric and phosphoric acids are eliminated by the kidney. In the filtrate, they are filtered off as salts of Na^+ and K^+. However, during the passage of the filtrate thorugh the tubule, the Na^+ is reabsorbed and replaced by H^+ or NH_4^+ secreted by the epithelial cells lining the tubule.

The H^+ ion is produced from the metabolic CO_2 combining with H_2O to form H_2CO_3 which dissociates to H^+ and HCO_3^-. The HCO_3^- is reabsorbed into blood along with Na^+ while the H^+ is secreted into the urine to replace Na^+.

The rapid conversion of CO_2 to H_2CO_3 and its further dissociation require the enzyme carbonic anhydrase. The renal tubular epithelium is rich in the enzyme (other tissues being the erythrocyte and parenchymatous cells of gastric mucosa).

The NH_3 is produced by the action of the enzyme glutaminase on glutamine The renal tubular epithelium is rich in this enzyme also. NH_3 formed by the action of the enzyme combines with H^+ (derived from H_2CO_3 to form NH_4^+ which will now exchange with Na^+ The exchange mechanisms are schematically represented in fig. 24-1.

Exchange of NH_4^+ for Na^+ does not alter the pH of urine. At the same time, it conserves sodium which is reabsorbed to combine with bicarbonate and reenter plasma. During conditions of acidosis, the excretion of NH_4^+ may increase five to ten times. Normally about 70 m. eq. of H^+ are excreted per day, of which only 30 m. eq. contribute to titrable acidity of urine. The rest is in the form of NH_4^+.

In renal tubular acidosis, there is an inability to excrete H^+ in normal amounts and leads to acidosis.

Tissue buffers: These also participate in regulation. During acidosis, cell K^+ comes out and is excreted in urine. In prolonged acidosis, there is marked depletion of intracellular potassium. Sodium and calcium from bone may also be depleted in prolonged acidosis.

The hydrogen ion concentration in the urine at pH 4.5 is about 1000 times that of plasma at pH 7.4.

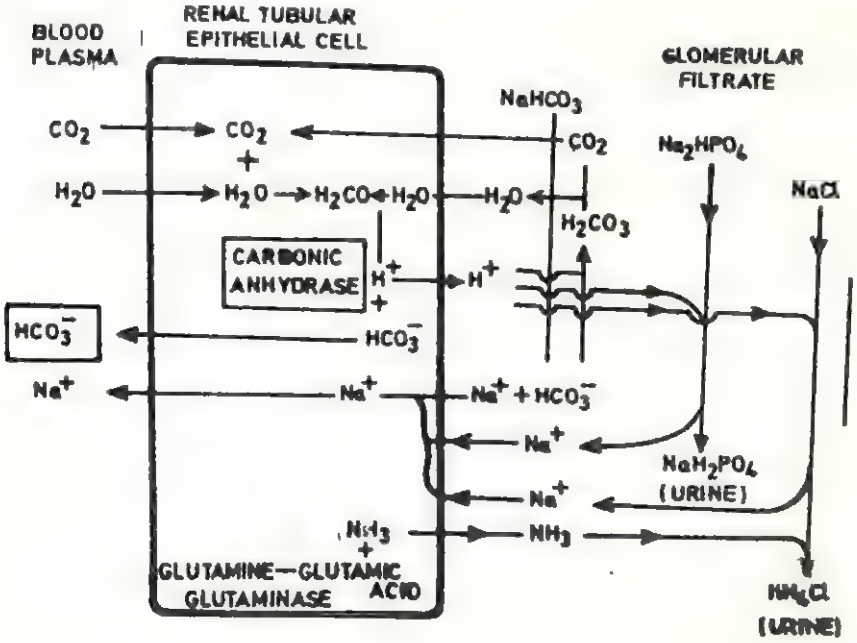


Fig. 24-1. Tubular function in acid-base balance

Diuretics: Increased urine output (diuresis) can be stimulated by several means.

(i) Increasing the concentration of solute will prevent complete re-absorption of the solute and there will be a proportionate amount of water of the filtrate also unabsorbed, thus increasing the urinary output. This is known as osmotic diuresis: Eg: injection of glucose solution.

Osmotic diuresis can be also induced by preventing reabsorption of the substances by administering drugs. Mercurial compounds inhibit reabsorption of NaCL and thus cause diuresis.

(ii) Carbonic anyhydrase inhibitors like acetazolamide (Diamox) inhibit the enzyme and prevent the secretion of H^+ ion from H_2CO_3 and thus cause an increase of sodium excretion and diuresis. There is also an increase of potassium excretion which exchanges with sodium in the distal tubule.

Kallikreins: These are peptidases produced by the distal tubular epithelial cells of the kidney. They are acidic glycoproteins. They can act on substances called *Kininogens* which are present in the α_2-globulin fraction of plasma proteins and convert them to *kinins*. (Such a conversion can also be brought about by trypsin and certain proteases present in snake venom.)

Plasma also contains kallikrein which is present in the precursor form as *'prokallikrein'*. This is an inactive form. Kallikrein can activate Hageman factor. It can also function as a chemotactic factor and can form bradykinin.

The kinins produced locally in the kideny probably function in regulating blood flow through the kideny and the excretion of salt and water. They do not have any systemic effects since they are rapidly degraded by kininases and excreted through urine.

Clinical abnormalities in tubular reabsorption:

1. *Renal glycosuria:* The tubule has diminished ability to reabsorb glucose. The Tm glucose is much less than the normal 350 mg/min. Hence glucose appears in the urine even at normal blood sugar levels. The renal threshold for glucose is lowered.

2. *Renal rickets* The threshold for phosphate is diminished with the result that the phosphate from filtrate is not reabsorbed but lost in urine and rickets is developed. This type of rickets does not respond to vitamin D therapy.

3. *Renal aminoaciduria:* Usually the ability to reabsorb diamino acids like cystine, arginine, ornithine and lysine is diminished. Occasionally aminoaciduria is also accompanied by glycosuria, hypophosphatemia and rickets. It is called de Toni-Fanconi syndrome.

Tests for renal function:

That volume of blood or plasma which contains the substance excreted in the urine in one minute is referred to as the 'clearance' of that substance.

1. *Inulin Clearance:* Inulin is a polysaccharide which is readily filtered by glomerulus, is neither secreted nor reabsorbed by the tubule and is not metabolized in the body. Hence the rate of its clearance gives a direct measure of the glomerular filtration rate (GFR). The inulin clearance or GFR in a healthy 70 kg adult man with 1.73 sq. metres body surface is 120 ml/min.

2. Endogenous creatinine also is readily filtered by glomerulus and not reabsorbed nor secreted. Hence the clearance of endogenous creatinine also is a measure of GFR.

Calculation of clearance values: If U is the concentration of the substance in urine, B its concentration in blood plasma and V the volume of urine per minute:

$$\frac{U \times V}{B} = C_{substance} \quad \text{(clearance of that substance).}$$

Urea clearance: The above calculation is applicable for calculating the clearance rate of urea, if the volume of urine secreted per minute is 2 ml or more. It is called the maximal clearance and is found to average 75 ml/min. If the urine volume per minute is less than 2 ml, urea clearance is found to vary as the square root of V.

$$C_{urea} \propto \sqrt{V} \text{ or } \frac{C}{\sqrt{V}} \text{ is constant.}$$

Substituting for C_{urea},

$$\frac{U \times V}{B} \times \frac{1}{\sqrt{V}} = K$$

$$\therefore \quad \frac{U \times \sqrt{V}}{B} = K$$

Hence the formula used for urea clearance when urine volume is below 2.0 ml/min. is $U\sqrt{V}/B$ and is called the standard clearance. A clearance value lower than the inulin clearance value indicates that some of the urea has been reabsorbed by the renal tubule (out of urea present in 120 ml filtrate originally formed, that contained in about 50 ml in case of maximal clearance has been reabsorbed).

Renal plasma flow (RPF): At low plasma concentrations para-aminohippurate (PAH) is found to be completely removed from plasma during one passage through the kidney. It is removed by glomerular filtration and tubular secretion. The clearance of PAH under these conditions, therefore, is a measure of the plasma flowing through the kidney per minute. RPF=PAH clearance (when its plasma concentration is below 2 mg/100 ml.)= 757 ml/ minute.

By estimating some of these clearances, one can detect whether the glomeruli are diseased or the tubule is diseased or whether the circulation through the kidney is affected and so on.

Composition of urine:

In a normal adult the volume of urine per day varies widely from about 500 ml. to as much as 2500 ml. The general characteristics are as follows:–Pale yellow or straw colored, clear, with aromatic odor when freshly voided.

1. *Specific gravity:* 1,003–1,030. One of the functions of the kidney is its ability to vary the concentration of the solutes from time to time as per the metabolic needs of the individual. A constant specific gravity within narrow limits near the lower extreme is indication of renal failure.

2. *PH:* Acidic to litmus (around pH 6.0). But highly variable from 4.9 to 8.0. Titrable acidity 20-40 ml. of 1.0 N acid/day. On standing for some hours, the urine turns alkaline due to the production of ammonia from urea by bacterial action.

3. *Solids in 24 hour urine:*

Chlorides as NaCL about 10 grams.
Ca^{++}, Mg^{++}, and iodine in small amounts.
Urea about 20–30 grams.
Creatinine 1.5 grams.
Ammonia 0.7 grams.
Uric acid 0.7 grams.

Sulphates, phosphates and oxalates and trace amounts of amino acids, vitamins, hormones and enzymes are also present.

An examination of the urine for abnormal constituents like glucose, ketone bodies, proteins and others is of immense diagnostic significance in diagnosing not only renal disease but also several systemic diseases like diabetes mellitus.

Abnormal Constituents of Urine:

1. *Glycosuria:* Normal urine does not contain reducing substances in urine that can be detected by the usual tests like the Benedict's. Presence of detectable amounts of glucose, fructose or pentose is therefore abnormal and is called glycosuria.

(i) *Glucosuria:* Excretion of glucose in urine may occur in emotional states and following anaesthesia or asphyxia. It also occurs in severe hyperthyroidism. The most common cause of glucosuria is however diabetes mellitus. The glucose concentration of urine in this condition may vary from 0.5 to 12.0%. There is also associated hyperglycemia. In conditions of diminished renal threshold for glucose due to a defect in the reabsorption of glucose by the renal tubule, glucosuria may occur even with normal blood glucose levels. The condition is called *renal glycosuria (glucosuria)*. 15% of pregnant women also show glucosuria without hyperglycemia.

(ii) *Fructosuria:* Excretion of fructose in urine may occur as a rare metabolic defect in the liver.

(iii) *Pentosuria:* May occur in normal people after ingestion of large amounts of fruits or fruit juices (alimentary pentosuria). In idiopathic pentosuria, L-xylulose is excreted in urine due to lack of L-xylulose dehydrogenase enzyme. The condition is apparently harmless.

(iv) *Lactosuria:* Excretion of lactose in urine occurs frequently in lactating women, but does not occur during pregnancy.

(v) *Galactosuria:* This is associated with the rare familial condition of *galactosemia*, a metabolic defect due to deficiency of the enzyme galactose-1-phosphate uridyl transferase. Galactose is excreted in urine.

2. *Proteinuria:* Trace amounts of protein, glycoprotein and mucoprotein are present in normal urine but are not detected by the usual clinical tests. Whenever abnormal amounts of protein are excreted in urine, albumin forms the major part of the protein. Hence proteinuria is more often called albuminuria. Proteninuria may occur in primary renal inflammatory diseases like glomerulonephritis and degenerative renal diseases like nephrotic syndrome. It may also occur secondarily in conditions like toxemias of pregnancy and congestive heart failure. Some, otherwise normal persons, show proteinuria on prolonged standing or walking. This is known as postural or orthostatic proteinuria.

Bence-Jones Proteinuria: Urine of individuals suffering from diseases like multiple myeloma, leukemia and Hodgkin's Disease contains a protein called Bence-Jones protein. It is precipitated on heating the urine to 50-60°C and redissolves when heated further to 100°C.

3. *Ketone bodies:* Acetone, acetoacetic acid and beta-hydroxybutyric acid may be excreted in urine in severe diabets mellitus and during prolonged starvation due to impairment of the carbohydrate metabolism. Ketone bodies may occur in urine following anaesthesia.

4. *Bile pigments and bile salts:* They occur in urine in conditions of hepatic or obstructive jaundice.

5. *Blood:* In inflammatory conditions or traumatic lesions of the kidney and in tumours of the urinary tract, urine may contain blood. The condition is called *hematuria*. Hemoglobin may be excreted in urine following excessive intravascular hemolysis as in blackwater fever (a manifestation of malaria) and in extensive burns.

6. *Porphyrins:* Excretion of more than normal amounts of porphyrins in urine is called *porphyria* and was considered under porphyrin metabolism.

Urinary Lithiasis:

Calculi or stones may form in the urinary tract due to the low solubility of some of the urine constituents. About a third of all such calculi contain calcium phosphate, calcium carbonate and magnesium-ammonium phosphate. Increase in the amount of calcium excreted or infections of the urinary tract and alkalinity of a urine predispose to their formation. One half of the renal calculi are made up of calcium oxalate as the main ingredient. Diets rich in spinach and cabbage contain large amounts of oxalates and aggravate the condition. Calcium oxalate stones may also occur in primary oxaluria, an inborn error of glycine metabolism. Uric acid stones are associated with gout. Cystine stones occur in cystinuria. Xanthine stones may occur occasionally due to defective purine metabolism.

The endocrine function of the kidney is considered in the chapter on 'Hormones'.

References:

Bland, J.H. (ed): Clinical Metabolism of Body Water and Electrolytes., Saunders, Philadelphia, 1963.

Cantarow, A. and Trumper, M: Clinical Biochemistry, 7th ed., Saunders, Philadelphia, 1975.

Lotspiech W.D.: Metabolic Aspects of Renal Function. Thomas, Springfield (ILL), 1959.

Pitts, R.F.: Physiology of the Kidney and Body Fluids., Yearbook Med. Pub., Chicago, 1963.

Smith, H.: The Kidney., Oxford Univ. Pr., 1951.

Smith, H.: The Principles of Renal Physiology., Oxford Univ. Pr., 1956.

Stanbury, J.B., Wayngaarden, J.B. and Fredrickson, D.S. (ed): The Metabolic Basis of Inherited Disease, McGraw-Hill, N.Y., 1972.

Thomson, R.H.S. and Wooton, I.D.P. (eds): Biochemical Disorders in Human Disease, 3rd ed., Churchill, London, 1970.

White, A.G.: Clinical Disturbances of Renal Function., Saunders, Philadelphia, 1961.

25

ASSESSMENT OF LIVER FUNCTION

THE liver is one of the most metabolically active tissues. It plays an important role in the following:

1. In blood circulation, it is a junction point between he portal and systemic circulations. In most inflammatory or fibrotic diseases of the liver there is an obstruction to the free communication of blood between the two systems leading to portal hypertension and leakage of the fluid into peritoneal cavity (ascites). Liver also plays a minor role as reticuloendothelial system on account of the Kupffer cells contained in it.

2 *Formation and excretion of bile:* The bile pigments, bile salts, cholesterol, heavy metals and the enzyme alkaline phosphatase are excreted through bile.

3. It has important functions in the metabolism of all the three principal dietary constituents–carbohydrates, lipids, and proteins, including the synthesis of plasma proteins.

4. It is an important organ in the detoxication mechanisms.

The liver has an immense reserve capacity and will be able to carry on most of the functions until a good portion of the liver is damaged. Hence a number of tests have been devised to test the several of its functions to enable an early diagnosis of its derangement.

Excretory functions

Bile pigments: The bile pigments are produced in the reticuloendothelial system, and are transported to the liver in loose combination with plasma albumin. In the humans, it is mostly bilirubin that is present in circulation. Only small amounts of biliverdin are present. In the liver, the insoluble pigments are conjugated with glucuronic acid to form the mono or diglucuronides, and rendered solube. They are now excreted through bile. In a test devised by Van den Bergh, the bilirubin forms a reddish compound with the diazo reagent (diazotized sulfanilic acid) and can be estimated colorimetrically. Bilirubin being insoluble in water, the color develops only when alcohol is added to the solution. This is known as the indirect Van den Bergh reaction and bilirubin is said to be the 'indirect reacting bilirubin.' The bilirubin of bile is in the conjugated form (bilirubin mono or diglucuronide) and is water soluble. Hence it develops red color directly on addition of diazo reagent within 1 minute. Hence it is known as the 'direct reacting bilirubin' or the '1-minute bilirubin.'

5. *Blood:* In inflammatory conditions or traumatic lesions of the kidney and in tumours of the urinary tract, urine may contain blood. The condition is called *hematuria*. Hemoglobin may be excreted in urine following excessive intravascular hemolysis as in blackwater fever (a manifestation of malaria) and in extensive burns.

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TABLE 25-1

	Hemolytic or pre-hepatic jaundice.	*Hepatic or parenchymatous jaundice.*	*Obstructive or post-hepatic jaundice.*
1.	*Cause:*		
	Due to excessive hemolysis, large amounts of hemoglobin are converted to bilirubin. Bilirubin production exceeds ability of liver to excrete it	The liver parenchyma due to inflammatory or fibrotic or other changes, is less efficient in removal of the normal amounts of bilirubin produced.	Due to an obstruction of biliary passages below the liver, bile regurgitates back into systemic circulation.
2.	*Type of Van den Bergh reaction:*		
	Indirect or delayed positive.	Biphasic.	Direct, one minute positive.
3.	*Pigment in circulation:*		
	Unconjugated bilirubin.	Mixture of conjugated and unconjugated bilirubin.	Conjugated bilirubin.
4.	*Urine:*		
	Negative for bile pigments and bile salts.	Positive for bile pigments and bile salts.	Positive for bile pigments and bile salts.
5.	*Urobilinogen in urine:*		
	Increased.	Increased or normal.	Decreased.
6.	*Feces:*		
	Dark colored due to increased stercobilinogen.	Pale due to decreased stercobilinogen.	Pale due to decreased stercobilinogen.
7.	*Steatorrhoea:*		
	Not present.	Present.	Present.

Normal blood plasma contains 0.2 to 0.8 mg. of bilirubin of the indirect type (unconjugated). The Van den Bergh reaction in 1 minute will be negative.

Jaundice: In certain conditions, the amount of bile pigments in plasma increases above the normal level. This may cause a deposition of the pigment in the skin, conjunctiva and mucus membranes of the mouth which will turn lemon yellow in color. This is known as jaundice. There is similar deposition of the pigment in all tissues and it is also excreted in

urine. The blood-brain barrier in the adult is impermeable to bile pigments. Hence adult brain is not stained in janudice. But in the new born infant, it is readily permeable and the child develops what is known as 'kernicterus.' Biliverdin has an intense green color, whereas bilirubin is yellowish brown. Occasionally there may be an increase in biliverdin, in which case the skin will show a greenish color instead of yellow. Biliverdin jaundice may occur in cases of biliary obstruction due to carcinoma and in severe hepatocellular failure.

Jaundice can be classified into three different types.

Hemolytic or prehepatic jaundice: In this condition, there is excessive hemolysis due to any cause, and excessive production of bile pigment. When this exceeds the capacity of the liver to excrete, unconjugated bilirubin accumulates in blood and causes jaundice.

Hepatic or parenchymatous jaundice: This is due to inflammation or fibrotic changes in the liver. The liver cell function is deranged and it fails to excrete the normal amounts of bilirubin brought to it and results in a rise of the unconjugated bilirubin in blood. In addition, the inflammatory and fibrotic changes cause certain amount of obstruction to the free passage of bile through the biliary passages and results in the reabsorption of some conjugated bilirubin into the hepatic lymphatics and veins. There is hence an elevation of conjugated bilirubin also in blood.

Obstructive or posthepatic jaundice: This is a result of obstruction to the bile ducts anywhere in their course and is mostly due to surgical causes (e.g. calculi, tumour etc.). Conjugated bilirubin from the biliary passages regurgitates into the lymphatics and hepatic veins. There is an increase of conjugated bilirubin in blood. The different features exhibited by these three types of jaundice are presented in Table 25-1.

Icterus index: Instead of diazotizing the serum bilirubin and measuring the red color produced, the yellow color of the jaundiced serum can be directly measured. This is known as 'icterus' index, normal being 4 to 6 units.

Urobilinogen in urine and feces: This is increased in hemolytic jaundice since a large amount of bile pigment is entering the intestines and is converted to the bilinogens. In hepatic and obstructive jaundice, little or no bilirubin enters the intestines. Hence there is a decrease in the bilinogens in the feces and urine. In hepatic jaundice, due to failure of the liver to convert stercobilinogen to bilirubin, there may be increased urobilinogen in urine. Normal excretion of urobilinogen in urine is 0–4 mg. in 24 hours.

Stercobilinogen in feces is 40–280 mg. in 24 hours.

Bilirubin in urine: Bilirubin does not pass into glomerular filtrate on account of its association with plasma albumin. Hence urine does not contain bile pigments in hemolytic jaundice. In the hepatic and obstructive jaundice blood contains the soluble glucuronides of bilirubin. Hence they readily pass through glomerular filter and bilirubinuria occurs.

In addition to the excretion of the physiological substances like bile pigments, liver has also the ability to excrete administered dyes like bromsulphalein (B.S.P.), rose bengal and exogenous bilirubin. If 5 mg. of BSP/kg body weight is injected intravenously, 90% of it is excreted in 30 minutes, and 95% in 45 minutes. The rate of removal is decreased in parenchymatous liver disease and also in circulatory failure.

Plasma alkaline phosphatase: This enzyme is excreted by the liver. In a normal person, plasma contains 3 – 11 units (King-Armstrong) of the enzyme per 100 ml. of plasma. In obstructive jaundice, this is very much increased.

Detoxication tests: Benzoic acid is conjugated with glycine to form hippuric acid in the liver and this is excreted through urine. In a normal individual, I.V. administration of 1.77 grams sodium benzoate results in excretion of 0.7 grams of hippuric acid in urine in one hour. Oral intake of 6 grams of sodium benzoate will result in excretion of 3 grams in urine within 4 hours.

Metabolic functions

Carbohydrate metabolism: Liver is concerned with synthesis and storage of carbohydrate as glycogen, its release by glycogenolysis as glucose and synthesis of glycoproteins.

Glucose tolerance: In hepatic disease, due to poor glycogen storage in the liver, there is fasting hypoglycemia. Following glucose ingestion, there is a steep rise in blood glucose to hyperglycemic levels due to decreased uptake as liver glycogen. But the muscle takes up glucose as usual and brings down the blood glucose level in a short time.

Galactose tolerance: Liver can convert galactose to glucose and thus enable its utilization. When liver function is below normal, the conversion does not occur and galactose is excreted in urine.

0.5 gm. of galactose/kg. body weight is injected I.V. in the postabsorptive state. In a normal person, there is no galactose in blood 75 minutes after the injection. In liver disease, blood will contain over 20 mg% of galactose.

Alternately, 30 grams of galactose is ingested by mouth. Urine collected for the next three to five hours contains less than 3 grams of galactose in the normal individual. It will be more in cases of liver failure.

Epinephrine and glucagon tolerance tests: Injection of epinephrine or glucagon stimulates hepatic glycogenolysis and causes rise in blood sugar by 40 mg% or more in one hour. In liver disease, due to lowered glycogen stores in that viscera, the rise in blood sugar will be much less.

Protein metabolism:

1. *Plasma protein changes:* A decrease in plasma albumin and a rise in the globulins occur in liver disease. This results in a reversal of the A/G ratio (from 2:1 it becomes 1:2 in extreme cases). The total proteins also fall.

2. *Prothrombin time:* The time required for clotting of a sample of plasma to which calcium and thromboplastin are added is called the prothrombin time since it is found to indicate the level of prothrombin in blood. Normal individuals show a prothrombin time of around 15 seconds. In liver disease, this is increased due to a deficiency of prothrombin, a protein exclusively synthesized by the liver.

In addition to quantitative changes of plasma proteins, qualitative changes occur in the lipoprotein and other fractions of plasma. These are tested empirically by certain procedures:

(a) *Thymol turbidity test:* The turbidity produced by 0.1 ml serum in 6.0 ml of the thymol reagent is a measure of liver function. Normally the turbidity is 0 to 4 units. In liver disease, this is increased.

(b) *Cephalin-cholesterol test:* A cephalin-cholesterol reagent shows turbidity and flocculation in hepatic disease on account of increased gamma globulin and decreased plasma albumin. The turbidity is read as 1+ to 4+. In liver disease, it is nearer 4+.

(c) *Zinc sulphate turbidity test:* Small amounts of the zinc salt will cause turbidity depending on amounts of gamma globulin present in the plasma. Normally it is 4 to 12 units of turbidity. It is much increased in liver disease.

Lipid metabolism: *Ester/free cholesterol ratio:* The liver is concerned in the synthesis, esterification, oxidation and excretion of cholesterol. Normal blood contains 150–250 mg of total cholesterol/100 ml. Of this, about two thirds (66%) is in the esterified form and the remaining one third in the free form. In obstructive jaundice, there is a gross increase in the total cholesterol content of blood without any alteration in the ester cholesterol/free cholesterol ratio. In hepatic jaundice, on the other hand, the total cholesterol level remains normal or is decreased and the ester/free ratio also is decreased, the ester forming less than two thirds of the total.

Enzymes in liver disease:

A number of enzymes like lactic dehydrogenase (LDH), glutamic-pyruvic transaminase (GPT) and glutamic-oxaloacetic transaminase (GOT) increase in blood plasma in liver disease. It may be on account of their release from the liver tissue which is damaged. Other enzymes which are increased are aldolase and phosphohexose isomerase and isocitric acid dehydrogenase (ICD).

Serum Iron: in hepatitis, the level of serum iron in the plasma reaches high levels.

Since no single test gives complete or conclusive evidence of liver damage, it is customary to do several tests and correlate the findings.

References:

Henry, R.J. et al (eds): Clinical Chemistry, 2nd ed. Harper & Row 1974.
Thomson, R.H.S. and Wooton, I.D.P. (eds): Biochemical Disorders in Human Disease, 3rd ed., Churchill, 1970.

26

ENERGY METABOLISM OR CALORIMETRY

THE mechanism of energy liberation by biological oxidation of carbohydrates, lipids and proteins had been considered in earlier chapters. It is necessary to have an accurate idea of the energy requirement of an individual in order to supply him sufficient food for the purpose.

Calorific values of food: When a substance is burnt in an atmosphere of oxygen in a closed cylinder, it generates heat. The heat generated can be measured by immersing the cylinder in a vessel containing known amount of water and finding the rise in temperature. The apparatus used for the purpose is known as a 'bomb calorimeter.' The unit of energy is a 'calorie' which is defined as the amount of heat required to raise the temperature of 1 gram of water by 1°C (specifically from 15°C to 16°C). This is the ordinary calorie and it is found too small a unit for measuring the energy value of foods. A unit thousand times the ordinary calorie called the kilocalorie or simply 'Calorie' (the word is spelt with a capital 'C') is used for this purpose. 'Calorie' in biological sciences always means a kilocalorie.

Food materials undergo combustion in the animal body and liberate energy in the same way as in a bomb calorimeter, but in a graded and continuous manner instead of in an explosive way. The calorific values of the principal food constituents are given in table 26-1.

TABLE 26-1.

Caloric, O_2 and CO_2 Equivalents of Carbohydrate, Fat and Protein

	Carbohydrate	*Fat*	*Protein*
Calories per gram	3.7–4.3	9.5	4.3
Litres CO_2 per gram	0.75–0.83	1.43	0.78
Litres O_2 per gram	0.75–0.83	2.03	0.97
Respiratory quotient	1.0	0.707	0.801
Caloric value per litre O_2	5.0	4.7	4.5

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On account of losses in digestion and absorption and other unaccountable factors, the calorific values are usually rounded off and said to be 4 calories per gram of carbohydrate and protein and 9 calories per gram of fat. In the case of protein, apart from losses in digestion

and absorption the oxidation is incomplete in the body since the excretory product, urea, is further oxidizable. Hence, while in the bomb calorimeter, protein yields 5.6 C per gram, in the body only 4 calories are realized.

The metabolism of these substances in the body liberates energy which is used for work and maintenance of body temperature. Ultimately the energy output can be measured as heat output from the body. In a healthy adult man, since the heat output has to be replaced by furnishing equivalent amount of calories in food, the calorie requirement can be measured by measuring the calorie output.

Direct calorimetry: The subject is kept inside an insulated chamber (Atwater-Benedict metabolism chamber) around which water is circulated at a known rate and known temperature. The rise in temperature of the water will indicate the energy output of the individual during that time. This is technically a difficult and costly procedure.

Indirect calorimetry: Information about energy output can also be obtained with almost the same degree of accuracy by indirect methods-*viz.*, measurement of CO_2 output and O_2 consumption and nitrogen excretion during a given time period.

Since each gram of urinary nitrogen represents 6.25 grams of protein metabolized in the body and since the calorific value of proteins is 4, the energy output on account of protein can be readily calculated. By conducting experiments in a bomb calorimeter with purified protein and other substances, the amount of oxygen utilized and CO_2 given off by a known amount of the substance can be also derived. If this portion of O_2 and CO_2 are subtracted from the total O_2 and CO_2 used in the experimental period, the amounts which are used or produced in the combustion of carbohydrates and lipids can be obtained. The complete oxidation of these substances can be represented as follows:

Carbohydrate: $C_6H_{12}O_6 + 6\,O_2 \longrightarrow 6\,CO_2 + 6\,H_2O.$

$$\text{Respiratory quotient} = \frac{CO_2 \text{ produced}}{O_2 \text{ utilized}} = 6/6 = 1$$

Lipid: For the oxidation of tristearin

$$2\,C_{57}H_{110}O_6 + 163\,O_2 \longrightarrow 114\,CO_2 + 110\,H_2O$$

$$\therefore \quad \text{R.Q.} = \frac{CO_2}{O_2} = 114/163 = 0.7$$

Conversely, it can be said that a R.Q. of 1.0 indicates that carbohydrate is being exclusively metabolized and R.Q. of 0.7 indicates that fat is exclusively being metabolized. For R.Q. values in between 0.7 and 1.0, varying proportions of carbohydrate and fat are being metabolized. The proportion of carbohydrate to lipid for any given R.Q. can be obtained by calculation or by reading off from a prepared nomogram (Fig. 26-1).

R.Q.	1.0	0.95	0.9	0.85	0.80	0.75	0.7	R.Q.
	100% carbohydrate metabolized						100% lipid metabolized.	

Fig. 26-1.

Knowing how much of protien (from urinary nitrogen estimation), carbohydrate and lipid (from non-protein R.Q.) are utilized during a given time, the energy supplied or expended can be readily calculated by multiplying with their calorific values. This is known as 'indirect calorimetry' and is technically simpler and cheaper and hence more widely applicable than direct calorimetry. In the indirect method, the subject may breathe in from atmospheric air and breath out into a closed container like the Douglas bag. This is known as the open circuit method. In the closed circuit system, the subject breathes in and out from same container. The CO_2 is absorbed by soda lime and oxygen slowly decreases in volume. The change in O_2 volume is measured and since the oxygen equivalent of the carbohydrate, lipid and protein are known (as indicated in the table 26-1) the total energy output for the given period can be calculated if the proportion of the three substances oxidized is known. This is known as the closed circuit system.

Basal Metabolism:

By standardizing the conditions under which the test is performed, it is possible to make the proportion of carbohydrate, lipid and protein oxidized constant for the given set of conditions. A subject is said to be under basal conditions if he is in the postabsorptive state (12–14 hours after the last meal), physically and mentally at rest, in a reclining position in bed, in a room whose temperature is about 25°C and the humidity of the air is comfortable. Under such conditions, energy output of the individual is to maintain respiration, circulation, muscle tone (skeletal and smooth muscle), functions of viscera like the kideny, liver, and brain and for the maintenance of the body temperature. The energy output under these basal conditions per unit time (one hour) is known as the 'basal metabolic rate' – abbreviated as B.M.R. A constant ratio of endogenous carbohydrate, lipid and protein are metabolized under such conditions. The R.Q. is 0.82 and each litre of O_2 consumed represents 4.825 calories of energy output. The O_2 consumption can be measured in a closed circuit system. The apparatus commonly used is the 'Benedict-Roth metabolism apparatus,' schematically represented in the figure 26-2.

Procedure: The apparatus consists of a cylindrical spirometer vessel closed at upper end and floating on water jacket below, to make an air-tight seal. The spirometer is filled with oxygen and is connected by passages regulated by valves – the outlet passage directly to the mouthpiece and the inlet passage through a soda-lime container, to absorb the CO_2 of the expired air. The subject, kept under basal conditions, is made to breathe by mouth by closing the nostrils and inserting the mouthpiece into his mouth. The respiratory excursions are transmitted to the floating respirometer which moves up and down in the water jacket

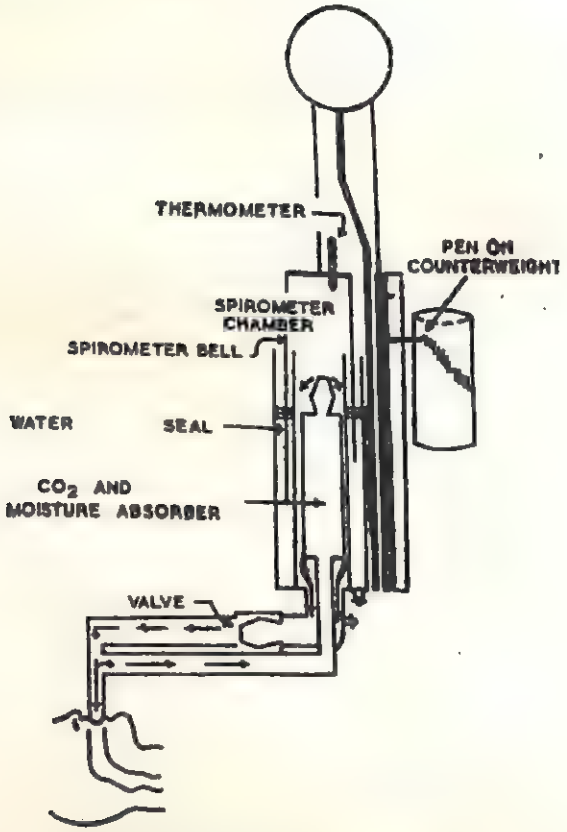

Fig. 26-2. Benedict–Roth apparatus for determination of B.M.R.

surrounding its lower portion. The movements of the respirometer in turn are transmitted to a pen connected to the top of the respirometer by a pulley and chain. The movements are recorded by the pen on a recording drum which is rotated by a mechanical or electrical clock work.

The test is usually run for a period of six minutes and the volume of oxygen consumed in that time is obtained from the tracing on the recording drum. From this, the oxygen consumption per hour is calculated. By multiplying this with 4.825, the energy output in calories per hour is obtained. Since B.M.R. is to be expressed as calories per square meter per hour, the energy output for hour obtained above has to be divided by the surface area of the individual which can be calculated from his height and weight by using Du Bois formula.

A healthy adult male has a B.M.R. of about 40 C per square meter of the body surface per hour. An adult woman has about 36 calories/square meter/hour.

Physiological variations of B.M.R:

1. *Surface area:* Since much of the basal metabolism is for the maintenance of body temperature and since heat loss is proportional to the surface area of the body, the B.M.R. is directly proportional to the body surface. Hence it is customary to express B.M.R. as calories per square meter per hour.

Surface area is difficult to measure directly in each case. However, based on actual measurements in a number of people, a formula relating the surface area to height and weight had been derived by Du Bois.

$$A = H^{0.725} \times 71.84. \times W^{0.425}$$

(Where A=surface are in sq.cm., H=height in cm. and W=weight in Kg). In practice even this calculation is avoidable by using a nomogram which relates the height and weight to the surface area.

2. *Age:* The B.M.R. is maximum at the age of 5 years. From then it steadily declines. There is a small increase during puberty.

3. *Sex:* Women have lower B.M.R. than men.

4. *Climate:* B.M.R. is higher in colder climates.

5. *Race:* The B.M.R. is higher in Western races, compared to the Orientals. This is independent of the effect of the climate.

6. *State of nutrition:* B.M.R. is lowered in conditions of malnutrition, starvation and wasting disease.

7. Drugs like epinephrine, benzedrine, caffeine, nicotine and alcohol increase the B.M.R.

Pathological variations in B.M.R.:

1. *Fever:* For every 1°C rise in the body temperature, there is an increase in B.M.R. by 7%.

2. Increased metabolic activity occurs in leukemias, polycythemia, cardiac failure, hypertension and laboured breathing (eg: asthma) and causes a rise in B.M.R.

3. *Endocrinal disturbances:* The most important factor which alters B.M.R. is the state of function of the thyroid. In fact, determination of B.M.R. is mainly used for the assessment of thyroid function. It is customary to express B.M.R. as the per cent of positive or negative change from the normal. Thus if the B.M.R. of an adult man of 28 years is 40 calories per

square meter per hour and the observed B.M.R. of a subject of that age is 50 calories per sq. m/hour, his B.M.R. is said to be $+\frac{50-40}{40} \times 100\%$ i.e.+25%.

If the observed B.M.R. is 30 C/sq. m/hr., then the B.M.R. is said to be $-\frac{40-30}{40} \times 100\%$ i.e.–25%.

In hyperthyroidism the B.M.R. is increased to + 75% or more. In hypothyroidism or myxedema, this is reduced to –40%. Variations between –10% to +15% are considered within physiological range and hence normal. The B.M.R. is also increased in Cushing's syndrome and acromegaly (hyperfunction of adrenal cortex and anterior pituitary) and decreased in Addison's disease (hypofunction of adrenal cortex).

Apart from its use as a diagnostic aid, knowledge of B.M.R. is essential in the calculation of calorie requirements and planning of nutrition for individuals as well as large communities and populations. The normal B.M.R. values for different age groups and the two sexes are tabulated in table 26-2.

TABLE 26-2.

Normal Basal Metabolic Rate (Calories per square meter).

Age in years.	*Male.*	*Female*
5 ..	53 ..	51
10 ..	49 ..	46
15 ..	46 ..	40
20 ..	41 ..	36
30 ..	39 ..	36
40 ..	38 ..	36
50 ..	37 ..	34
60 ..	35 ..	33

27

NUTRITION

FOOD is a prime necessity of life. It supplies the energy required for the work done by the individual and also for growth and reproduction. The constant synthesis of tissues taking place during periods of growth and even in the fully grown adult requires the supply of the basic building materials such as monosaccharides, fatty acids and amino acids. Minerals and vitamins also have to be supplied through food. The study of the requirements of these various substances and how the requirements can be met through diet is known as 'nutrition.'

Calorie requirements: To maintain caloric balance in an adult, it is necessary to supply enough food to replace the calories expended per day.
This includes–

1. A supply of basal requirements (B.M.R.).

2. Supply of calories to meet the extra requirement caused by specific dynamic action (S.D.A.) or calorigenic action of foods.

3. Supply for physical activity over and above the basal requirements.

4. During periods of growth or convalescence, extra provision has to be made to meet the synthesis of tissues and weight gain.

1. *B.M.R.*: This has been already discussed in detail. For an adult man of 70 kg body weight and surface area 1.7 sq.m. the basal requirement will be $1.7 \times 40 \times 24 = 1632$C or say 1600 C/day.

2. *Specific dynamic action or calorigenic action of foods:*

If an individual, whose B.M.R. is 1600 calories, is fed with just enough food to provide 1600 calories and is kept under basal conditions (except that he is not under post-absorptive state), it is found that his energy output has increased beyound the basal output of 1600. The increase varies with the type of food that has supplied the calories. If the calories are exclusively derived from protein, there is an increase in the output by 30% (i.e., 1600+480 calories), if exclusively from carbohydrate the increase is 5-6% and if exclusively from fat the increase is 4% over the basal level. On a mixed diet,however, the increase is 10–12%. This stimulant action of food on the metabolism is known as the specific dynamic action (S.D.A.) or calorigenic action of food. The mechanism of stimulation is not clear.

It was at one time considered to be on account of the energy required for the digestion and absorption of food. But it is no longer acceptable since administration of glucose or amino acids intravenously also has calorigenic effect. A part of the increase is probably on account of the synthetic reactions like urea formation and lipogenesis. There is also most probably a general stimulus to all metabolic reactions. The liver appears to be an important site for the calorigenic effect.

To provide for this increase in metabolism, ăn extra provision of about 10% of basal requirement has to be made. Thus, for a 70 kg man with B.M.R. of 1600 C, another 160 C have to be added on this account.

3. *Physical activity:* This introduces a highly variable element in the calculation of energy requirements. Table 27-1 gives a rough indication of the requirements for some of the typical activities.

TABLE 27-1-A.

Additional Calorie Requirements over the Basal For Different Types of Work.

Sitting	..	35	Calories/hour.
Standing	..	40	" "
Tailoring, typing, writing	..	70–80	" "
Household work	..	100–120	" "
Walking, moderate speed, light games	..	250	" "
Fast walking, running, manual labour, strenuous games, swimming	..	300–550	" "
Getting up stairs	..	1000	" "

Oxygen consumption by some viscera in the human body as % of total oxygen consumption.

Name of viscera.	*At rest.*	*During Light work.*	*Heavy work.*
Skeletal muscle ..	30	70 (210)	86 (688)
Abdominal viscera ..	25	9 (27)	3 (24)
Heart ..	11	9 (27)	5 (40)
Kidneys ..	7	2 (6)	1.5 (12)
Brain ..	20	6 (18)	2 (16)
Skin ..	2	2 (6)	1.5 (12)
Others ..	5	2 (6)	1 (8)
	100	100 (300)	100 (800)

The oxygen consumption per minute increases 3 fold in light work and 8 fold in heavy work. The increase (actual amounts) are shown within brackets.

TABLE 27-1-B.

Calorie Requirements per day

	Adult man (55 Kg. Wt.)	*Adult woman (45 Kg. Wt.)*
Sedentary Work ..	2,400 C	1,900 C
Moderate Work ..	2,800 C	2,200 C
Heavy Work ..	3,900 C	3,000 C
	During pregnancy + 300 C.	
	During lactation + 700 C.	

TABLE 27-1-C.

Minimum and Maximum Weights as prescribed by Life Insurance Corporation of India.

Height in inches ..	57	60	63	66	69	72
Minimum wt. in lbs. ..	75	84	93	102	111	123
Maximum wt. in lbs. ..	130	142	154	169	184	202

Total calorie requirements: A complete knowledge of the hours of activity, type of activity, hours of rest and sleep besides height, weight, age and sex are required to calculate the daily calorie requirements.

For an average Indian male medical student, aged 22 years, height 162 cm (5'-4') and weight 55 kg. (120 lbs.), the surface area is 1.55 sq. meters. His B.M.R. is 41 C/sq m/hr.

1. His basal requirements/day = 41 × 1.55 × 24 = 1525 C
2. S.D.A. 10% of basal requriements = 152 C

3. (a)	8 hours moderate work (walking, playing simple games like badminton, shuttle etc.,), extra 100 C/hour	800 C
(b)	8 hours sedentary activity like sitting and reading, writing etc., extra 40 C/hour	320 C
	Total	2797 C
	or say	2800 C

Man coefficients: In dealing with large communities, it is not practicable to calculate on an individual basis. Taking the requirement of an adult man as unit, the requirement of others is worked out as shown in table 27-2.

TABLE 27-2.

			Coefficient.
Adult male			1.0
Adult female		..	0.9
Adolescent	12–21 years	..	1.0
Children	9–12 years	..	0.8
"	7–9 years	..	0.7
"	5–7 years	..	0.6
"	3–5 years	..	0.5
"	1–3 years	..	0.4

The man-equivalents or coefficients of the entire community can be calculated and the total calorie requirements arrived at.

For infants from 1 month to 12 months old, the calorie requirements vary from 200 to 800 calories per day.

Planning of diets:

In the planning of suitable diets for the individual or for the community, not only the quantity of food and its calorific value have to be considered, but of equally great importance is the quality of food.

Protein, lipid and carbohydrate are the three proximate principles of food and are the main suppliers of calories.

Protein requirements:

It is the most important constituent of food since it is essential for synthesis of protoplasm, enzymes and hormones and is required for the supply of the essential amino acids which cannot be synthesized in the body.

According to Rose, the following are the daily requirements of essential amino acids for a young man to maintain nitrogen balance.

Amino acid.		*mg/kg*
Tryptophan	..	7
Phenylalanine	..	31
Lysine	..	23
Threonine	..	14
Valine	..	23
Methionine	..	31
Leucine	..	31
Isoleucine	..	20

The above quantities recommended are on the liberal side for the maintenance of nitrogen balance. Arginine and histidine are not required. But prolonged histidine deficiency results in impaired hemoglobin synthesis and eczematous skin lesions in infants. The requirements can be considerably brought down if adequate amounts of the nonessential amino acids are provided in the diet. Thus methionine requirements will be less if cysteine is added to the diet. Phenylalanine requirments can be reduced if tyrosine is present in adequate amounts in diet. Hence, a balanced mixture of amino acids as occurs in natural proteins is better than a mixture of essential amino acids only in maintaining normal health.

The absolute need of protein is for the supply of essential amino acids. Thus, if enough calories are supplied by fats and carbohydrates, the protein supplies can be cut down to quite low levels (protein-sparing action of carbohydrate and fat).

Not only the total amount, but the type of protein taken is also important. Broadly speaking, there are two main forms of proteins – animal protein and vegetable protein. The digestibility and absorbability vary from protein to protein. Animal proteins are generally better digested and absorbed. Since they contain the essential amino acids in the same proportion as present in the tissues of the body, they are also more readily and more completely utilized for synthesis of tissue protein. Vegetable proteins are relatively low in lysine, methionine and tryptophan.

In man no disease caused by deficiency of a single amino acid has been described. In growing rats, deficiency of any single amino acid leads to failure of growth, loss of appetite, and certain specific disorders like hepatic necrosis in deficiency of sulfur containing amino acids, cataracts in histidine or tryptophan deficiency and anemia and hypoproteinemia in deficiency of most of the amino acids.

A total reduction of the protein content (hence amino acid content) of diet results in the manifestation of methionine deficiency first, since the daily requirements of that amino acid are the highest. Fatty infiltration of the liver and cirrhosis will result. Addition of choline to the low protein diet will prevent the damage to the liver. Severe protein depletion will result in acute hepatic necrosis. This can be prevented by adding cysteine to the diet.

Long standing protein depletion of a mild degree results in the development of anemia, hypoalbuminemia and edema in rats. Malnourished human subjects also show similar conditions which are hence attributable to protein deficiency. These individuals are also prone to a high incidence of peptic ulcer.

Biological value of protein: This is defined as the per cent of the absorbed protein nitrogen that is not excreted in urine (hence retained in the body). A high biological value therefore indicates a high degree of utilization of the amino acids of the protein. Animal proteins like liver, meat, egg and milk protein have high biological values compared to vegetable proteins like those of cereals, pulses, and vegetable (see table 27-3). The biological value of a mixture of proteins is more than the average of their individual biological values. This is because, the deficiencies of amino acids in individual proteins are made up by the amino acids in the other proteins. Thus the biological value of vegetable protein is improved if it is taken along with animal protein. Of the daily protein supply, it is recommended that one third to one half be derived from animal proteins like egg, meat and milk.

TABLE 27-3.

Biological values of some proteins.

Animal protein		*Vegetable protein*	
Egg, whole	94	Barley	71
Egg, white	83	Maize	60
Cow's milk	85	Ragi	89
Milk powder	83	Rice	86
Mutton	60	Wheat	67
Beef	69	Bengalgram	76
Pork	77	Blackgram	64
Fish	70–80	Greengram	51
Liver	77	Redgram	74
		Soya bean	54
		Cashew-nut	72
		Ground-nut	57

The minimum protein required for nitrogen balance in Indian adults is 0.51 to 0.66 gm (average 0.57 gm.) per kg. body weight. This does not much differ from the requirement observed in Western subjects. The protein, in the case of Indian, is mostly derived from vegetable sources. Since such dietary protein is only about 85% digestible and has a low biological value of around 60, the net utilization of vegetable protein will be about 65% only. Hence, in order to supply 0.57 gm. protein, an allowance of 1.0 gm. per Kg. has to be made. The I.C.M.R. group has hence recommended an intake of 1.0 gm./Kg. for an adult. During pregnancy and lactation, an additional 10–20 gm. of protein has to be added per day. Infants and children require much larger amounts, 1.5–2.0 gm./Kg.

Protein deficiency:

Lack of a single amino acid does not occur naturally, but can be produced experimentally. Hepatic necrosis, anemia, cataract, hemorrhagic necrosis of the kidney, hypoproteinemia, fatty liver, hepatic cirrhosis, hypospermia and anestrus are some of the conditions caused by depriving some of the essential amino acids from animal diets.

Low protein diets cause anemia, hypoalbuminemia, and edema in experimental animals. If the diet is also deficient in choline, the animal develops fatty liver and cirrhosis. Populations on low protein diet show a higher incidence of peptic ulcer.

Infants who are weaned early from mother's milk (may be on account of the arrival of the next child too soon) and fed on protein-poor diets develop a disease called 'Kwashiorkar.' They are retarded in physical and mental growth and show anemia, hypoproteinemia, cheilosis, stomatitis, conjunctivitis, edema and fatty liver. There is also an atrophy of the acinar portion of pancreas resulting in indigestion, diarrhoea, steatorrhoea, which all further aggravate the protein deficiency. Kidneys are also damaged, resulting in increased excretion of amino acids in urine. The children are very prone to intercurrent infections. Mortality in untreated children is quite high.

In economically poor populations, protein lack is also usually associated with a general calorie deficit, 'protein-calorie malnutrition,' and is an even more serious condition.

Fat requirements:

Fat is a concentrated source of calories (9 calories/gram) and its use in cooking makes food more palatable and less bulky to take. Apart from it, it provides the essential fatty acids and the fat soluble vitamins. A minimum of 15 gms. per day are recommended. To guard against the development of hypercholesterolemia and atherosclerosis, it is further recommended that fat should not supply more than 30% of total dietary calories. Unlike in the case of proteins, vegetable fats are superior to animal fats because they contain more of the poly-unsaturated fatty acids which are essential. They are also cheaper. Ground-nut, gingely, mustard and safflower oils are some of the vegetable oils in common use. Butter, ghee and the fat present in animal meat are the usual sources for animal fats. The effects of deprivation of essential fatty acids in diet are described in the chapter on lipid metabolism.

Carbohydrate requirements:

Carbohydrate containing foods (cereals and pulses mainly) are the cheapest and hence form the main source of calories in the average diet. The balance of calories (after supply by the protein and fat) have to be met by carbohydrates. Thus, a 2,800 calorie requirement for a 55 kg. adult man has to be met as follows:–

Proteins	55 grams	× 4	..	220 calories
Fats	55 grams	× 9	..	495 calories
Carbohydrates	521.25 grams	× 4	..	2085 calories

Approximately 500 grams of carbohydrate have to be provided. These are supplied in the form of cereals (rice, wheat, maize, corn etc.), pulses (different grams), jaggery and cane sugar. They contain varying amounts of the B-complex group of vitamins also.

In addition to calorie requirements met by the three proximate principles of diet, minerals and vitamins are also to be supplied. Calcium, phosphorus and iron are among the minerals likely to be deficient in Indian diets. Calcium and phosphorus can be obtained best through milk and milk products and green leafy vegetables. The FAO/WHO committee suggested an intake of 400–500 mg/day for an adult. The ICMR group suggests similar intake for Indian adults. In pregnant and lactating women, an intake of 1.0 gm. per day is recommended. Children require as much as an adult. The recommended daily allowance for children is 400 – 700 mg.

Iron: The WHO and the National Research Council (USA) have recommended 10 mg/day for adult man and 12–15 mg/day for an adult woman. The requirement rises to 20 mg/day during pregnancy and lactation. Adolescent boys and girls need 15 mg/day. Most Indian diets contain more than these amounts. But the rate of absorption is poor on account of the large amount of phytate in the predominantly vegetarian diet. The ICMR group has therefore recommended a daily intake of 20 mg. by adult man and 30 mg. by adult woman. During pregnancy and lactation, this has to be further increased to 40 mg. Adolescent boys require 25 mg. and girls 35 mg. per day. Children in the lower age groups require 15–20 mg/day.

Among the vitamins, the requirements of fat-soluble A will be adequately met if the recommended amount of fat is taken in diet. Vitamin D requirements of children are provided by milk and milk products and eggs. Supplementation is best done by fish liver oils, which are rich in these vitamins. The B-complex vitamins are present in cereals and pulses. Ascorbic acid is obtained from citrus fruits, milk and germinating seeds.

Details of the requirements and sources of the minerals and vitamins were already discussed in the relevant chapters.

Some of the common Indian foods and their composition are presented in table 27-4.

The daily allowances of several nutrients recommended to the average Indian by the Nutritional Expert Group, 1968 are presented in table 27-5.

Alcohol in Human Nutrition:

Alcohol is consumed in the form of drinks and appetizers.

Beer: This is obtained by fermentation of malt. Different brands contain 4–8% alcohol by volume. They also contain small amounts of unaltered sugar (maltose) and dextrins. Beer provides about 50 calories per 100 ml.

Wines: They are manufactured by fermentation of fruit juices, mainly grape juice. Alcohol content varies from 10 to 20 vols, per cent. Traces of sugars and several organic acids like malic, tartaric and succinic acids and alcohols other than ethyl alcohol are also present. 'Cider' is a wine from apple juice and has rather low alcoholic content (3–8 vols. %) Wines supply about 75 calories per 100 ml.

Gin and Whisky: They are manufactured by distilling beer. Their alcohol content varies from 30–50 vols%.

Brandy: This has similar alcohol content and is obtained by distilling the wines. This group provides about 220 calories per 100 ml.

In addition to alcohol, they all contain small amounts of organic esters, essential oils and flavouring agents which gives them their distinctive taste and flavour. They also exert varying degrees of stimulant action on the stomach and intestines and modify the rate of absorption of the alcohol.

Taken on an empty stomach, about 20% of the ingested alcohol is rapidly absorbed from the stomach itself, and the rest from the intestine. The presence of food in the stomach slows the rate of absorption. The action on brain is also subdued if the blood sugar level is high. Due to the rapidity of absorption, peak concentration is reached in blood within an hour of ingestion of the alcoholic drink.

90% of the alcohol is oxidized to CO_2 and H_2O. The remaining 10% is removed through respiration and urinary excretion. The kidney cannot excrete much alcohol because the alcohol in the glomerular filtrate is rapidly reabsorbed by the tubule. Alcohol concentration in urine is only about 20% more than its concentration in blood at that time.

A single dose of 2 ounces of whisky which contains about 24 grams of alcohol causes a blood concentration of 30 mg per 100 ml. At this concentration, only a sense of euphoria occurs in most individuals. At a level of 150 mg% many will show signs of intoxication. Even in those individuals where intoxication signs are not manifest, tests will reveal a decrease in muscular coordination etc. Blood levels of 200 mg% can be positively considered to be intoxication levels. Levels of 300–400 mg% result in stupor and coma. Levels of 500 mg% are fatal.

A drug called 'antabuse' causes inhibition of the enzyme aldehyde dehydrogenase and causes accumulation of acetaldehyde in the liver. This makes the subject feel very sick even after a small drink and thus weans him out of the habit.

TABLE 27-4

Per 100 Gms	Calories	Pro-teins	Carbo-hydrates	Fat	Calcium	Phos-phorus	Iron	Thia-mine Mi-crogram	Ribo-flavin Micro-gram	Niacin	Ascor-bic Acid	Caro-tene Mic-rogram	Vita-min A I.U.
		Gms	Gms	Gms	Gms	Gms	mg			mg	mg		
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
Cereals and pulses													
Rice (Milled) ..	350	6.8	78	0.5	0.01	0.16	3.1	60	60	1.9	..	..	..
Rice (Hand pounded) ..	350	7.5	77	1.0	0.01	0.19	3.2	210	160	3.9	..	2	..
Rice (Parboiled, Milled) ..	350	6.4	79	0.4	0.01	0.14	4.0	210	50	3.8	..	..	..
Wheat (Whole) ..	350	11.8	71	1.5	0.04	0.31	4.9	450	170	5.5	..	64	..
Wheat Flour (Atta) ..	340	12.1	69	1.7	0.05	0.36	11.5	490	290	4.3	..	29	..
Wheat Flour (Refined) ..	350	11.1	74	0.9	0.02	0.12	2.5	120	70	2.4	..	25	..
Maize ..	340	11.0	66	3.6	0.01	0.35	2.0	420	100	1.8	..	90	..
Barley ..	335	11.5	70	1.3	0.03	0.22	3.0	470	200	5.4	..	10	..
Ragi ..	328	7.3	72	1.3	0.34	0.28	6.4	420	190	1.1	..	42	..
Bengal Gram Dhal ..	372	20.8	60	5.6	0.06	0.33	9.1	480	180	2.4	1	129	..
Black Gram Dhal ..	350	24.0	60	1.4	0.15	0.39	9.1	420	370	2.0	..	38	..
Green Gram Dhal ..	350	24.5	60	1.2	0.08	0.41	8.5	720	150	2.4	..	49	..
Peas, dried ..	315	19.7	57	1.1	0.08	0.30	5.1	470	190	3.4	..	39	..
Redgram Dhal ..	335	22.3	58	1.7	0.07	0.30	5.8	450	190	2.9	..	132	..
Soya Bean ..	432	43.2	21	19.5	0.24	0.69	11.5	730	390	3.2	..	426	..
Green, leafy vegetables													
Amaranth ..	45	4.0	6.1	0.5	0.40	0.08	25.5	30	300	1.2	99	5520	..
Cabbage ..	27	1.8	4.6	0.1	0.04	0.04	0.8	60	90	0.4	124	1200	..
Drumstick leaves ..	92	6.7	12.5	1.7	0.44	0.07	7.0	60	50	0.8	220	6780	..
Gogu (Red Sorrel) ..	56	1.7	10.0	1.1	0.17	0.04	5.0	70	390	1.1	200	2900	..

TABLE 27-4 *(Contd.)*

Per 100 Gms	Calories	Pro - teins	Carbo-hydrates	Fat	Calcium	Phos-phorus	Iron	Thia-mine Mi-crogram	Ribo-flavin Micro-gram	Niacin	Ascor-bic Acid	Caro-tene Mic-rogram	Vita-min A I.U.
		Gms	Gms	Gms	Gms	Gms	mg			mg	mg		
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
Mint ..	48	4.8	5.8	0.6	0.20	0.06	15.6	50	260	1.0	270	1620	..
Spinach ..	26	2.0	2.9	0.7	0.07	0.02	10.9	30	260	0.5	28	5580	..
Roots and tubers													
Beet root ..	43	1.7	8.8	0.1	0.20	0.06	1.0	40	90	0.4	10	Trace	..
Carrot ..	48	0.9	10.6	0.2	0.08	0.53	2.2	40	20	0.6	3	1890	..
Potato ..	97	1.6	22.6	0.1	0.01	0.04	0.7	100	10	1.2	17	24	..
Tapioca ..	157	0.7	38.1	0.2	0.05	0.04	0.9	50	100	0.3	25	..	..
Nuts and seeds													
Cashewnut ..	596	21.2	22.3	46.9	0.05	0.45	5.0	630	190	1.2	..	60	..
Coconut, fresh ..	444	4.5	13.0	41.6	0.01	0.24	1.7	50	100	0.8	1	..	..
Groundnut ..	570	25.3	26.1	40.1	0.09	0.35	2.8	900	130	20.0	..	37	..
Gingely seeds ..	563	18.3	25.0	43.3	1.45	0.57	10.5	1010	340	4.4	..	60	..
Mustard seeds ..	541	20.0	23.8	39.7	0.49	0.70	17.9	650	260	4.0	..	162	..
Safflower seeds ..	356	13.5	17.9	25.6	0.24	0.82	..	..	..	..	..	..	..
Fruits													
Apple ..	59	0.2	13.4	0.5	0.01	0.01	1.0	120	30	0.2	1	..	..
Banana, ripe ..	116	1.2	27.2	0.3	0.02	0.04	0.9	50	80	0.5	1	78	..
Gooseberry ..	53	1.8	11.1	0.2	0.01	0.07	2.0	50	20	0.3	49	1428	..
Cashew fruits ..	51	0.2	12.3	0.1	0.01	0.01	0.2	20	50	0.4	180	23	..
Dates, dry ..	317	2.5	75.8	0.4	0.12	0.05	7.3	10	20	0.9	3	26	..

Effect of cooking on diets:

The process of cooking makes the food more palatable and tasty and in most cases more easily digested. The cellulose cell wall of vegetable cells is broken and the starch is readily digested. Proteins of the collagen type are converted to gelatin-like material which is digestible. The harmful effects of avidin present in egg white are lost when egg is cooked. So also the trypsin inhibitor in soya bean is removed on cooking. The biological value of soya bean protein increases on cooking. But there are also some losses of important nutrients. The habit of cooking the foods in excess of water and discarding the excess water removes good amounts of protein and water soluble vitamins. Even prolonged washing of the cereals before cooking removes much of the water-soluble vitamins and minerals.

Addition of soda (sodium bicarbonate) to vegetables and pulses during cooking causes loss of vitamins, while addition of acidic materials like tamarind helps in preserving them. Boiling of milk causes loss of the vitamin C contained in it. Frying in open pan causes loss of vitamin A present in the oils used.

Some practical ways of improving diets:

A balanced diet for one day for an adult man is given below–

Cereals	..	400 grams.
Pulses	..	90 "
Green leafy vegetables	..	120 "
Tubers and roots	..	90 "
Other vegetables	..	90 "
Fruits	..	90 "
Milk	..	300 "
Sugar or jaggery	..	60 "
Vegetable oil, ghee	..	60 "
Fish and meat	..	90 "
One egg	..	50 "

Such a diet will be however too costly for an average man. To provide the principal protective foods – proteins, minerals and vitamins – cheap substances like groundnut cake (after expelling oil from ground-nut), and soya bean meal can be tried. Some of the cereals like ragi contain more protein and minerals than rice. It is therefore advantageous to include some of these cereals in diet. The cheapest of vegetables like amaranth and spinach are more nutritious than some of the costlier ones and can be used with advantage. They supply minerals and also vitamin A precursor – carotene. Fresh whole milk is costly, but skimmed milk powder is cheaper and is an excellent source of protein of high biological value. It is also a good addition to children's diets. The cheaper fruits like tomato and banana are as nutritious if not more nutritious than the costlier apples and grapes. The State also can help by supplying enriched bread with added B vitamins and amino acids like lysine.

Infant feeding: Breast milk is the best for an infant upto 6 months. Where this is inadequate or not available, milk substitutes of like composition have to be supplied. The composition of milk from different sources is given in table 27-6.

TABLE 27-6.

	Calories	Moisture	Protein	Fat	Carbohydrates	Mineral	Calcium	Phosphorus	Iron	Vit. A	Vit. B_1	Riboflavin	Niacin	Vit. C
Cow	67	87.5	3.2	4.1	4.4	0.8	0.12	0.09	0.2	174	50	190	0.1	2
Buffalo	117	81.0	4.3	8.8	5.0	0.8	0.21	0.13	0.2	160	40	100	0.1	1
Goat	72	86.8	3.3	4.5	4.6	0.8	0.17	0.12	0.3	182	50	40	0.3	1
Human	65	88.0	1.1	3.4	7.4	0.1	0.03	0.01	0.2	137	20	20	–	3

Cow's milk or buffalo's milk can be fed to the infant after suitable dilution to bring down the protein and fat content and by adding sugar, preferably lactose itself, to raise the sugar content of diluted milk to that contained in human milk.

Metabolic changes in Starvation:

Studies were made in fasting human volunteers by G.F. Cahill, Jr. and others in Boston.

Within 24 to 48 hours after commencement of the fast, liver glycogen falls to about 10% of its normal concentration and remains at that level almost during the entire remaining period of fasting. The blood glucose level remains relatively constant at about 80 mg. per 100 ml. for about 4 weeks. Within 24 to 48 hours from the commencement of the fast, increased mobilization of depot fats from the abdominal and subcutaneous areas starts. The blood ketone levels are increased and respiratory quotient comes down from the normal 0.82 to a level of 0.7 or slightly above. In a few days, urinary nitrogen excretion (mainly urea) starts increasing. The need for protein breakdown even when adequate fat stores are available is to supply glucose to the brain by gluconeogenesis. 20% of energy expenditure in the body under basal conditions is on account of brain. It requiires about 140 grams of glucose per day. 100 grams of protein can supply about 57 grams glucose by gluconeogenesis. Only the glycerol part of lipids can form glucose. The erythrocytes also metabolize mostly glucose by glycolysis. The lactic acid produced can be reconverted to glucose by the liver. Hence the erythrocyte metabolism does not pose much of a problem.

The depletion of the body proteins follows a definite pattern. The earliest proteins to be depleted are the enzymes of the gastrointestinal secretions like the gastric, pancreatic and intestinal secretions. Since there is no food intake, this is an appropriate and natural method of adaptation. The heptaic enzymes concerned in processing the absorbed nutrients to plasma proteins, lipoproteins and lipids are also lost early. Next, the muscle starts losing the structural proteins as well as glycolytic enzyme proteins.

In the first week of fasting, about 100 grams of body protein are metabolized per day. But in course of time and by the end of 4 to 6 weeks of fasting, the protein metabolism falls to as low as 12 to 15 grams a day. The brain develops the ability to utilize ketone bodies as alternate fuel to glucose. β-Hydroxybutyrate is the preferred fuel. This enables the individual to fast much longer periods by expending body fat stores and conserving body protein.

Once the fat stores are depleted, the condition of the fasting person takes a rapid turn to the worse. The basal metabolism has to be entirely met by protein and the condition becomes critical. The skeletal muscle mass supplies most of the protein at this stage.

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1. C, Gopalan and B.S. Narasinga Rao, 1971.
"Dietary Allowances for Indians."
Nutritional Research Laboratories, Indian Council of Medical Research (Hyderabad).

2. C. Gopalan, B.V. Rama Sastry and S.C. Balasubramaniyan, 1971.
"Nutritive Value of Indian Foods."
National Inst. of Nutrition, Indian Council of Medical Research (Hyderabad).

References:

Albanese, A (ed): Protein and Amino Acid Nutrition, Acad. Pr., N.Y., 1959.

Albritton, E.C. (ed): Standard Values in Nutrition and Metabolism, Saunders, Philadelphia, 1954.

Brock, J.F.: Recent Advances in Human Nutrition, Little Brown., Boston, 1961.

Consolazio, C.F., Johnson, R.E. and Mark; E.: Metabolic Methods, Mosby, St. Louis (Missouri) 1951.

Cooper L.F., Barber, E.M. and Mitchell, H.S.: Nutrition in Health and Disease, Lippincot, Philadelphia, 1960.

Davidson, S. Passmore, R.: Human Nutrition and Dietetics, 4th ed., Williams & Wilkins., Baltimore, 1969.

Gopalan, C. and Narasinha Rao, B.S.: Dietary Allowances for Indians., Nutr. Res Lab., ICMR, Hyderabad, 1971.

Gopalan, C., Rama Sastry, B.V. and Balasubrahmaniyan, S.C.: Nutritive Value of Indian Foods, Natl. Inst., Nutr., ICMR, Hyderabad, 1971.

McDivit, M.E. and Mudambi, S.R.: Human Nutrition, Principles and Applications in India, Prentice Hall, New Delhi, 1973.

Natl. Acad. of Sci.: Recommended Dietary Allowances, Report of Food and Nutrition Board, 7th ed., Publication No. 1694, Natl. Res. Council, Washington, D.C. 1968.

Sardesai, V.M. (ed): Bio-Chemical and Clinical Aspects of Alcohol Metabolism, Thomas, Springfield (ILL), 1969.

Williams, S.R.: Nutrition and Diet Therapy, Mosby, St. Louis (Missouri), 1969.

Wohl, M.G. and Goodhart, R.S. (eds): Modern Nutrition in Health and Disease: Lea & Febiger, Philadelphia, 1968.

28

HORMONES

THE term hormone was first applied by Bayliss and Starling in 1902 for 'Secretin' produced by intestinal mucosa.

Hormones are substances produced by specialized tissues called the endocrines or the ductless glands and liberated into the blood stream to be carried to a remote tissue or viscera called the target organ on which they exert characteristic physiological effects. Like vitamins and enzymes the hormones are effective in minute amounts. Though the name hormone indicates that its action is to stimulate ('hormaein' in Greek means 'to excite'), not all of them are excitatory. They differ from enzymes in that they are not all of them protein in nature and as a rule do not act on the endocrine gland which has produced them. In fact, the hormones in many cases, act by influencing the enzymes.

Mode of action of hormones: The gross physiological effects of the hormones are known for a long time, but cellular and molecular levels of their action are little understood even now. A few possible modes of hormone action are mentioned below:–

(a) *Induction or repression of genetically controlled enzyme synthesis:* Certain isotopically labelled hormones, when administered parenterally, are found to be more concentrated in the cell nuclei of the target organ. They may combine with a specific receptor protein. The receptor protein may be in the cytosol or nucleus. The protein-hormone complex formed influences the genes to increase the formation of mRNA concerned with the specific enzyme synthesis. Adrenocortical and other steroidal hormones and the thyroid hormones act in this way. Inhibitors of RNA synthesis such as actinomycin-D are observed to block the physiological actions of these hormones.

The influence may also be excercised at a lower level–the ribosomes. They do not then effect the synthesis of mRNA, but they alter the rate of function of the mRNA at the ribosomal level. Growth hormone functions in this manner.

(b) *Action on the cell membrane:* The action of some hormones appears to be initiated at the membrane level. The hormone binds to specific receptors on the cell membrane. This binding results in the alteration of the permeability of the membrane to specific substances like amino acids, glucose and ions. The entry of these substances into the cells will bring about qualitative and quantitative changes in the cell metabolism. Most protein hormones like insulin and the catecholamines seem to act in this way.

(c) *Alterations in the Levels of Cyclic AMP, the second messenger:* Hormones can be broadly divided into two groups:–1. Those which exert their effect on the target tissues immediately and whose effects are short lived. Epinephrine, glucagon, insulin, gastrin, secretin, parathormone, calcitonin and most of anterior and posterier pituitary hormones are of this type. 2. Those which elicit their response at a much slower rate and whose effects last longer. The steroidal hormones mostly belong to this category.

The first group can be considered to be 'messengers' which carry information from the endocrine gland to the target tissue. They act by binding to a 'Regulatory Site' (R) on the outer surface of the cell membrane (see fig. 28-1A). On the inner surface of the cell membrane adjoining the regulatory site is a 'Catalytic Site' "C" which

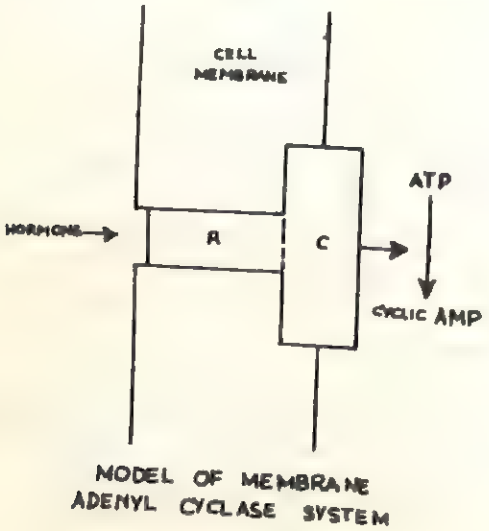


Fig. 28-1. A.

contains the adenyl cyclase enzyme. The interaction between the hormone and the receptor site will result in the stimulation of the adenyl cyclase at the catalytic site. the adenyl cyclase will convert A.T.P. to 3', 5'-cyclic A.M.P. (see Fig. 28-1 B & C) which enters the cytosol and in turn stimulates certain protein kinases which will phosphorylate certain enzyme molecules. Some enzymes, on phosphorylation, become active (*eg.* phosphorylase), while some others become inactive (*eg.* glycogen synthetase). Certain reactions are therefore stimulated while certain others are inhibited by the cyclic A.M.P. Cyclic A.M.P. can therefore be considered as the 'Second Messenger.' The hormone, therefore, need not enter the cell to produce its effects. Also, a small number of hormone molecules bound to the cell membrane can stimulate the production of a large number of cyclic A.M.P. molecules in the cell–an amplifying or magnifying effect.

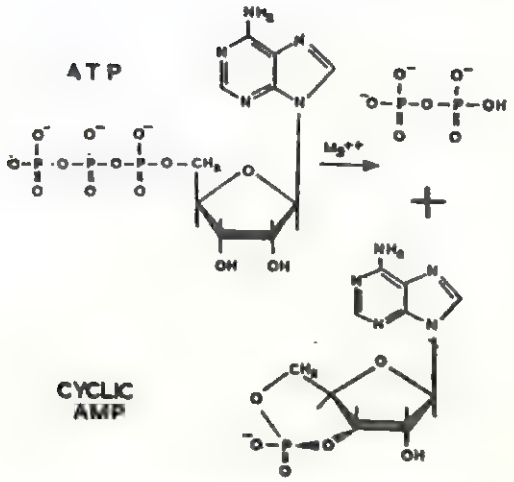


Fig. 28-1. B

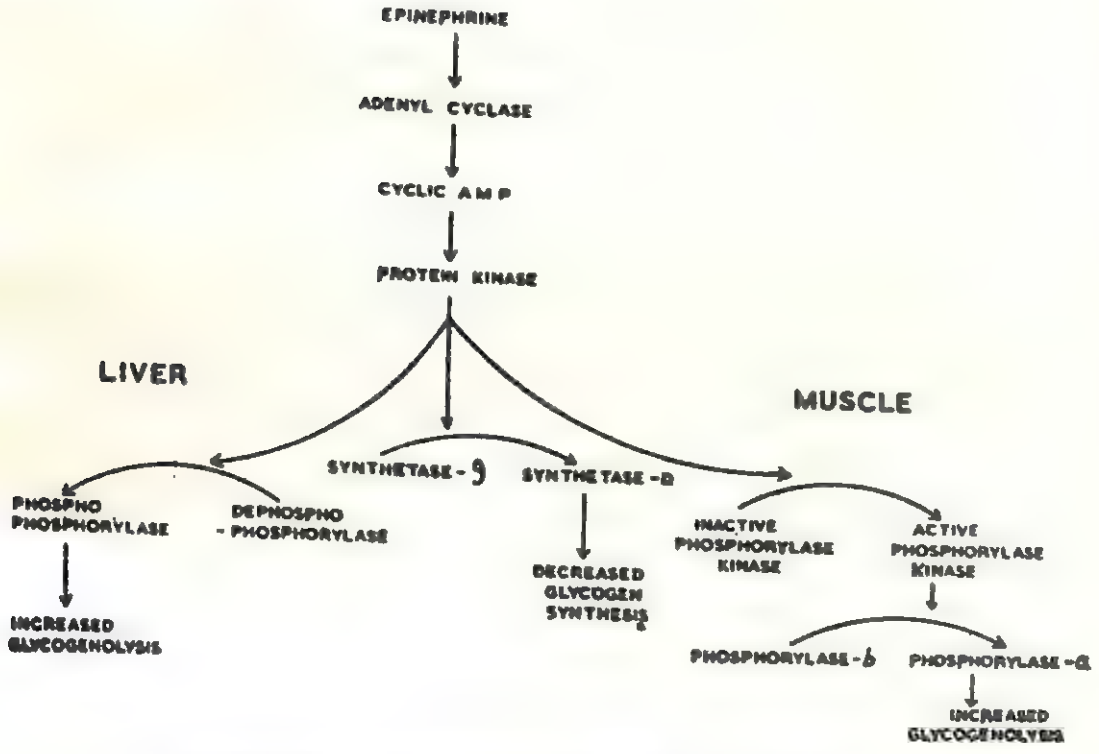


Fig. 28-1. C Influence of cyclic A.M.P. on liver and muscle glycogen.

Cholera toxin stimulates adenyl cyclase directly and causes production of excessive amounts of cyclic A.M.P. in the mucosal cells of the intestinal epithelium. There is excessive secretion of litres of electrolyte-rich fluid by those cells.

The cyclic A.M.P. is inactivated by the action of a specific enzyme 'Phosphodiesterase' which converts cyclic A.M.P. to ordinary A.M.P. Inhibitors of the phosphodiesterase like theophylline and caffeine thus prolong the action of the hormones which act through cyclic A.M.P. Insulin, on binding to the receptors on the target cell membrane, causes a decrease in cyclic AMP level, unlike epinephrine and glucagon. However, it acts on another nucleotide–GTP–and accelerates its conversion to cyclic GMP. The cyclic AMP and cyclic GMP seem to act in a reciprocal relationship. This is known as the Yin-Yang Hypothesis.

(d) *Role of Calcium:* Calmodulin is a protein with a molecular weight of 17,000. It is heat labile and acidic and has 4 binding sites for calcium. It is distributed throughout cytoplasm and is also associated with the cell membrane and certain enzymes. The binding of calcium to calmodulin will result in conformational changes leading to changes in the activity of enzymes and membranes. The action of many protein hormones is inhibited in the absence of calcium. The calcium may enter the cell from extracellular fluid or may be released within the cell itself from tissue-bound calcium. Protein hormones increase the permeability of extracellular fluid calcium into the cell. Cyclic AMP releases bound intracellular calcium. Thus calcium may play the role of the final messenger or the *third messenger* in eliciting the actions of certain hormones. The release of stored hormones from secretory granules also requires calcium (eg. insulin).

(e) *Prostaglandins as modulators of hormone action:* Prostaglandins seem to modulate the action of hormones rather than act as hormones themselves. The effect of PGE_1 on the breakdown of fat in adipose tissue was studied in detail. Epinephrine, glucagon, ACTH and TSH stimulate lipolysis. PGE_1 strongly inhibits the lipolytic effect of these hormones. The rise of the intracellular cyclic AMP by these hormones is prevented. Probably the action is by inhibiting adenyl cyclase of adipose tissue cells.

The different mechanisms of action mentioned above are interdependent and may be operating together in most cases.

Hormones can be broadly classified into three groups–

1. Steroidal hormones, produced by the adrenal cortex, testis and ovary.
2. Protein hormones, produced by pancreas, parathyroid, pituitary and the gastrointestinal mucosa.
3. Amino acid derivatives, produced by thyroid and adrenal medulla.

GASTROINTESTINAL HORMONES

Gastrin: HCL of gastric juice or the substances present in or derived from food stimulate the pyloric mucosa which secretes gastrin. This is absorbed into blood and carried to the parietal cells of the gastric glands and stimulates them to secrete a HCL-rich gastric juice.

Two hormones–gastrin I and gastrin II have been identified. Both are small polypeptides containing 17 amino acids. The c-terminal 4 amino acids seem to be most important for the function (-trp-met-asp-phe). A synthetic preparation - 'Pentagastrin' containing five amino acids (including the above four) is used clinically to stimulate gastric secretion.

Vagal stimulation, acetylcholine, and intake of foods, particularly if protein-rich, cause a release of gastrin. Glycine is a potent stimulus. Gastrin-producing pancreatic tumors, arising probably from the D cells, cause high gastrin production, increased HCL secretion and high incidence of peptic ulcers. It is described as Zollinger-Ellison syndrome.

Secretin: It is formed by the duodenal and jejunal mucosa. It is also a polypeptide hormone containing 27 amino acids. 14 of these are identical to those found in glucagon. It has a glucagon-like action in increasing cardiac output and lipolysis. It is secreted on stimulation by ingested food and acid chyme from stomach. It stimulates the secretion of a bicarbonate-rich watery secretion by pancreas.

Cholecystokinin-pancreozymin: Cholecystokinin and pancreozymin were once considered to be two distnict hormones. Now they are recognized to be one single hormone.

It is a polypeptide containing 33 amino acids, the c-terminal five amino acids being the same as for gastrin. Much of the activity resides in the c-terminal 8 amino acids. It stimulates the secretion of the pancreatic enzymes and also causes contraction of the gall bladder. It has also gastrin-like and secretin-like actions and stimulates release of both insulin and glucagon from pancreatic islets. That is why oral glucose is more effective in stimulating insulin release than parenterally administered glucose. It is liberated from the duodenal and jejunal mucosa.

Other gastrointestinal substances having hormone-like activity: Several factors are secreted by the gastrointestinal mucosa which are polypeptides having hormonal actions. These are called *'candidate hormones'*.

Gastric Inhibitory Polypeptide (GIP): Inhibits the gastric acid secretion and motility of the stomach.

Vasoactive Intestinal Polypeptide (VIP): Secreted by the mucosa of the small intestine and colon. It also inhibits gastric acid secretion and inhibits the motility of the stomach and gall bladder. It stimulates pancreatic and intestinal secretions.

Motilin: Stimulates gastric motility.

Enteroglucagon: Stimulates glycogenolysis.

Chymodenin: Stimulates chymotrypsin secretion by the pancreas.

Bulbogastrin: Secreted by the duodenal bulb and inhibits gastric HCL secretion.

Urogastrone: Extracts from normal urine, when administered parenterally, inhibit secretion of HCL by the gastric mucosa. The substance responsible for this action is named 'urogastrone.' Its exact nature and its relationship to other gastrointestinal hormones and its physiological role are not known.

HORMONAL FUNCTION OF THE KIDNEY–RENIN

The famous experiments of Goldblatt and others have shown that occlusion of arterial blood supply to the kidney by any method leading to renal ischemia will stimulate the renal cortex to liberate into the blood stream a substance called 'renin' which is a proteolytic enzyme. Blood contains 'angiotensinogen,' an alfa-2 globulin, produced in the liver. Renin acts on angiotensinogen and liberates from it a decapeptide (containing 10 amino acids) called 'angiotensin-1' which is further acted upon by an enzyme present in blood and converted to 'angiotensin-II' which is an octapeptide containing only eight amino acids. Angiotensin-II is a powerful pressor substance about 200 times as active as norepinephrine. It increases the force of cardiac contraction, constricts the arterioles and causes contraction of smooth muscle. Angiotensin-I has similar, but much feebler, actions.

Anatagonists to angiotensin II - its production or its action - are used in the treatment of hypertension.

1. Saralasin: The first and eighth amino acids of angiotensin are substituted by other amino acids. It acts as a competitive inhibitor by occupying the receptor sites.

2. Propanolol: Antagonizes the beta-adrenergic catecholamines and prevents the release of renin.

3. Teprotide: Blocks the conversion of angiotensin I to angiotensin II by combining with the converting enzyme.

The kidney also elaborates another proteolytic enzyme called 'angiotensinase' which is capable of inactivating angiotensin by hydrolyzing it.

It is doubtful whether this renal pressor system has any role in the normal person. But in diseases which cause a decrease in circulation through the kidney (*eg.* glomerulonephritis, perinephritis) this system is responsible for the causation of a persistent hypertension. The kidney also produces *'kininogen,'* a substance having an antihypertensive effect. It also produces two hormones having the effect of stimulating red blood cell production by bone marrow–erythropoietin and erythrogenin.

Its role in activating vitamin D by adding to the 25-hydroxycholecalciferol (the -OH at C-25 is added by liver) one more -OH at C-1 has already been mentioned under vitamin D.

It has also an enzyme system capable of hydroxylating at C-24 which will result in the formation of an inactive derivative of the vitamin D–24, 25-dihydroxycholecalciferol.

Some of the hormones like insulin, glucagon and aldosterone are destroyed by the kidney.

THYMUS

Thymus was once considered to be only a vestigial organ. Its importance in the immune system of the body has come to light only in recent years. Embryonic stem cells from the yolk sac and the liver of the fetus and the stem cells of the bone marrow of the adult migrate to the thymus and proliferate there. They may be called the 'prethymocytes.' They acquire their immunological properties in the thymus. A small number of them (about 5%) leave the thymus and reenter circulation as the T-cells. The remaining 95% are destroyed in the thymus itself. The T-cells are of three types in the human. About 40% of them have receptor sites for Ig_M and are called T_μ cells. Another 10% have receptors for Ig_G and are called the $T\gamma$ cells. The remaining 50% do not have any receptors for any of the immunoglobulins. The $T\gamma$ cells are the killers and the $T\mu$ cells the helpers.

Thymus is now incriminated in the etiology of myasthenia gravis. It is said to be caused by the individual developing autoimmunity against muscle proteins. A deficiency of thymus-dependent, cell-mediated immunoresponse is said to be the cause of diseases like sarcoidosis, and lepromatous reaction in leprosy.

Thymus involutes with age. Its size is maximal at birth, begins to shrink at puberty and is generally not discernible after middle age. This may be the reason for the inability of the elderly to devolop resistance to infections to which they were not already exposed during childhood and to neoplasms.

The thymus is also said to elaborate certain hormones which influence the development and maturation of certain types of lymphoid cells (see under 'lymphocytes' in the chapter on 'Blood and the Body Fluids'). Several hormones were identified–thymosins, thymopoietins, thymic factors, lymphocyte stimulating hormones, thymosterin etc. They are all active in stimulating T-cell immunological responses mainly and have also minor side effects from which their names are derived. Calcitonin is also secreted by the thymus.

Thymectomy in experimental animals soon after birth results in failure of the animals to grow, wasting and death in a few weeks or months. Lymphoid tissues and lymphoid cells of blood are deficient. Immunoglobulin response is poor.

Inadequacy of thymus in early life in the human is associated with diminished humoral as well as cell-mediated immunological response and in some cases agammaglobulinemia.

Hypothalamus

Hypothalamus occupies a pivotal place in the control of synthesis and release of most hormones. In response to specific neural messages from the peripheral tissues, it secretes infinitesimally small quantities of hormones called *'Releasing Factors.'* They are passed down the nerve fibres to the anterior pituitary gland (*eg.* TRF-thyrotropin releasing factor).

also a stimulant for insulin secretion. Glucose stimulates calcium uptake and production of cyclic AMP by the beta cell. Localization of calcium in certain areas of the beta cell may be necessary for the secretion of insulin.

Tolbutamide, one of the oral antidiabetic drugs, also stimulates insulin release independent of alterations in glucose level and even when glucose metabolism is blocked by antimetabolites.

Amino acids 'leucine and arginine' can also stimulate insulin secretion.

The role of fatty acids in stimulating insulin production is not clear.

Many of the hormones like growth hormone and glucocorticoids stimulate insulin secretion through their hyperglycemic effect. Glucagon seems to stimulate insulin production not only by causing hyperglycemia but also by increasing the cyclic AMP in the pancreas. The cyclic AMP stimulates glucose metabolism and thus supplies citric acid cycle intermediates which stimulate insulin secretion.

Epinephrine inhibitis insulin secretion in spite of the hyperglycemia.

In vitro, calcium and potassium stimulate insulin secretion while magnesium is inhibitory.

Glucose taken by mouth is a better stimulus for insulin secretion than when taken by injection. This is attributed to the action of the intestinal hormones like pancreozymin, secretin and glucagon-like substances which are released when glucose is administered by mouth. They seem to stimulate insulin secretion.

Vagal stimulation increases insulin secretion.

Metabolism of insulin: Insulin is believed to be transported in the plasma bound to a specific insulin transporting protein. Insulin is degraded primarily in the liver and kidney by the enzyme "glutathione-insulin transhydrogenase" which cleaves the -S-S-linkages to -SH, thus separating the A and B chains. The hydrogen is derived from glutathione. The A and B chains undergo further hydrolysis by the enzyme '*insulinase*'. The half-life of plasma insulin is only 7 to 15 minutes.

Mode of action of insulin: Muscle, adipose tissue and liver are the major sites of its action. It is also active on the lens and leukocytes. It has little action on the metabolism of the renal tissue, erythrocytes and the gastrointestinal tract.

1. *Extrahepatic tissues:* Insulin gets absorbed on to the cell membrane and probably is linked by an opening and reformation of one of the disulfide linkages in the molecule with the membrane protein. It facilitates the transport of glucose across the cell membrane. This being the rate limiting step in glucose metabolism, insulin promotes all subsequent metabolic pathways--glycogenesis, glycolysis and HMP-pathway. This results in production of more acetyl-coenzyme A through glycolysis and more NADPH + H^+ from HMP-pathway which in turn favour fatty acid synthesis and lipogenesis in the adipose tissue.

Insulin stimulates intracellular transport of all sugars which have same configuration as glucose in carbons 1, 2 and 3 (eg: arabinose, xylose and galactose). Fructose (which differs in C_2 by having a keto group) does not depend on insulin for its transport into the cell.

Insulin also sitmulates the uptake of amino acids by the cells. As in the case of glucose, uptake of amino acids is also independent of their subsequent utilization in the cell. Insulin not only facilitates transport of amino acids into cells, but also stimulates their incorporation into protein. The effect is exerted at the ribosomal level.

Insulin is said to stimulate the activity of the enzymes hexokinase and glycogen synthetase. The effects are not demonstrable in vitro on purified enzyme preparations.

It is said to stimulate oxidative phosphorylation in mitochondria of muclse. In the adipose tissue, it antagonizes epinephrine and glucagon action by suppressing fatty acid release by that tissue. The action may be two fold–

(i) By increasing glycolysis, more glycerophosphate is made available from glucose. This is used for synthesis of triglyceride from fatty acids.

(ii) By reducing the level of cyclic AMP in adipose tissue it inhibits lipolysis and fatty acid release.

Insulin stimulates the entry of Na^+, K^+ and phosphate into adipose tissue cells. This action is independent of glucose utilization by those cells.

2. *Liver:* The liver cell membrane is freely permeable to glucose and the concentration of glucose within the cells is same as in the extracellular fluid surrounding it. Hence the action of insulin on cell membrane has no relevance to liver cell.

Insulin is said to act on the hepatic cell by increasing the synthesis of the glucokinase, phosphofructokinase, and pyruvate kinase – enzymes concerned in glycolysis – and by repressing the synthesis of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose – 1, 6 – diphosphatase and glucose – 6 – phosphatase enzymes concerned in gluconeogenesis.

The net result of these actions is a decreased glucose output by the liver due to an increased glycolysis and decreased gluconeogenesis. The cyclic AMP level of liver is decreased. Insulin bound to the cell membrane will stimulate the action of membrane bound cyclic AMP-phosphodiesterase and inhibit the stimulatory effects of the hormones on adneyl cyclase activity (epinephrine and glucagon). The result is a decrease in the cyclic AMP levels in the cell.

The action on hepatic enzymes probably depends on a single factor – action on protein kinases – resulting in the formation of dephosphorylated enzymes (glucagon and epinephrine cause formation of phosphorylated enzymes).

Insulin is thus an anabolic hormone causing increased carbohydrate metabolism, glycogen formation, lipid synthesis, amino acid uptake and protein synthesis.

Assay of insulin: A unit of insulin is the amount required to reduce the blood glucose level of a normal 24-hour-fasted 2 kg. rabbit from 120 mg. to 45 mg/100 ml.

Several methods are used for assay of insulin:

1. Measuring the hypoglycemic effect in rabbit.
2. Uptake of glucose by rat diaphragm.
3. Uptake of glucose by epididymal fat pad in the rat.
4. Immunological assay using antibodies developed against insulin.

Assay of insulin by measuring the uptake of glucose by rat diaphragm or epididymal fat pad gives higher values compared to more accurate assays using antibodies or radio-immunoassay. The former is hence termed "insulinlike activity, (ILA)." The excess over actual insulin is called bound, nonsuppressible (by antibodies) or atypical insulin. Much of this activity is said to be due to "somatomedin," a factor released from liver by growth hormone activity.

Serum insulin levels by radioimmunoassay are about 25 microunits per ml in the normal fasting individual.

Diabetes mellitus:

Diabetes in Greek means 'siphon.

Mellitus means 'sweet.

This is a metabolic disorder occuring as a result of an insulin lack or a surplus of insulin antagonists leading to a relative insulin lack. It is characterized by hyperglycemia and glycosuria. Three types of the disease are described–

1. *Juvenile type:* Occurs in children and is due to an absolute deficiency of insulin.

2. *Maturity onset type:* Here insulin is secreted by the pancreas but it somehow fails to exert it's full action on the peripheral tissues. It is usually associated with obesity.

3. *Secondary diabetes:* Here there is hyperfunction of one or other of insulin anatgonists leading to a relative insufficiency of insulin eg: acromegaly (excess of growth hormone); Cushing's disease (excess of glucocorticoids); hyperthyroidism etc.

Experimental diabetes can be produced by total pancreatectomy or selective destruction of the beta cells by injecting alloxan, a pyrimidine derivative, which is highly and selectively toxic to the beta cells. Injection of insulin antibodies can also cause diabetes. Phlorhizin causes glycosuria by decreasing renal tubular reabsorption of glucose (renal glycosuria) and decreasing the renal threshold for glucose.

Glucagon is one of the contributory factors in the etiology of diabetes mellitus. Its blood levels are elevated in severe diabetes with ketoacidosis. The alfa cells seem to be insensitive to the high blood glucose levels in diabetes and continue to secrete large amounts of glucagon.

Somatostatin, a hypothalamic factor inhibiting the release of growth hormone, also inhibits the release of glucagon and is in experimental use as an adjunct to insulin in the control of severe diabetes mellitus. Somatostatin is also secreted by the D (delta) cells of the pancreas and the gastric mucosa. It is a small peptide containing 14 amino acids.

In a moderately severe early diabetes mellitus the following features are present–

1. Hyperglycemia.

2. Glycosuria.

3. Loss of weight due to increased breakdown of fat and tissue protein.

4. Increased production of ketone bodies by the liver and their incomplete utilization by tissues leading to their accumulation in blood (ketosis) and elimination in urine (ketonuria).

5. Lowering of the pH of blood due to circulating keto acids (acidosis).

6. Dehydration due to elimination of large amounts of water with glucose in urine.

7. Negative nitrogen balance due to conversion of more amino acids into glucose (increased gluconeogensis).

8. Increased levels of lipid, fatty acid and cholesterol in blood (lipemia).

9. Increased tendency to develop cataract in the eye and atheromatous and arteriosclerotic lesions of blood vessels.

The life expectancy of an untreated diabetic is very much shortened on account of the above.

Insulin resistance: Diabetics undergoing treatment with insulin may develop, in course of time, antibodies to insulin. They bind to the insulin injected and make it ineffective, requiring an increase in the insulin dosage. A change in the brand of insulin may be effective in such cases. The development of resistance can be avoided by injecting highly purified insulin preparations.

A second type of insulin resistance is due to presence of antibodies against the insulin receptors. The antibodies bind to the insulin receptors, thus denying them access to insulin. Insulin fails to exert its normal action due to nonavailability of receptors.

Hyperinsulinism: This may occur on account of islet-cell tumours involving the β-cells. There is overproduction of insulin resulting in spontaneous attacks of hypoglycemia associated with sweating, tremors and fainting attacks which are relieved by ingestion of glucose or a lump of sugar.

GLUCAGON

Glucagon, the hyperglycemic, glycogenolytic factor of the pancreas (H.G.F.), is secreted by the alfa cells of the pancreas. The hyperglycemic effect of some pancreatic extracts due to presence of this hormone was first noted by Kimball and Murlin in 1923.

It is a polypeptide with a molecular weight of 3485. The polypeptide consists of 29 amino acid residues arranged in a straight chain. Unlike insulin, glucagon crystals are free from zinc.

In addition to the alfa cells of pancreas, the gastric and duodenal mucosa also elaborate glucagon-like substances. The structure of glucagon closely resembles that of secretin. Ingestion of carbohydrate in food stimulates the release of intestinal glucagon which in turn stimulates the pancreatic β-cells to secrete insulin. Glucagon is transported in blood in the free state and is destroyed in the liver by the enzyme 'glucagonase.' The stimulus to glucagon production is lowering of blood glucose levels.

Glucagon stimulates hepatic adenyl cyclase enzyme thereby leading to an increase of cyclic AMP level in that viscera. This in turn stimulates the dephosphophosphorylasekinase enzyme resulting in the reactivation of phosphorylase. The final result of glucagon action is thus an increase in the active form of phosphorylase and increased breakdown of liver glycogen resulting in a blood sugar rise (hyperglycemia). The effect is seen within minutes of glucagon administration and the hyperglycemia lasts for an hour or more following a single injection.

The hormone has no action on the muscle phosphorylase. Hence the blood pyruvate and lactate do not show any change.

Glucagon inhibits hepatic synthesis of protein from amino acids and synthesis of fatty acids and cholesterol from acetate. The amino acids are used for increased gluconeogenesis. There is alo increased lipolysis and ketone body formation in the liver.

Glucagon increases glomerular filtration rate (GFR) and augments the urinary excretion of sodium, chloride, potassium and phosphate.

It also stimulates lipolysis in adipose tissue. All these three actions are antagonistic to insulin action. Hence diabetes produced by pancreatectomy (where insulin and glucagon are both absent) is less severe than that produced by selective destruction of beta cells by toxic substances like alloxan (where insulin is absent but glucagon is still present).

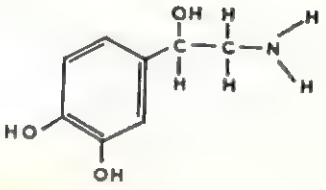
THE ADRENALS

The adrenal gland consists of a medulla and a cortex and each of them produces different hormones.

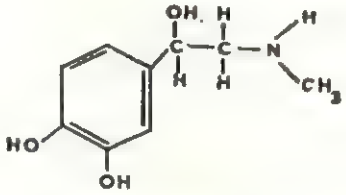
ADRENAL MEDULLA

This arises from the sympathetic portion of the autonomic nervous system. It produces two hormones – norepinephrine and epinephrine (noradrenaline and adrenaline). The physiological action of epinephrine closely resembles that of sympathetic stimulation. Adrenal medulla, though it has several physiological functions, is not indispensable for life.

Chemistry: They are catecholamine derivatives of tyrosine. Their formation from phenyl alanine and tyrosine had been already considered under the metabolism of those amino acids. Norepinephrine is formed first and is then converted to epinephrine by methylaion of the side chain. Epinephrine is the main hormone produced by the medulla. Norepinephrine is found principally in the sympathetic nerves. The naturally occurring hormones are L-isomers and are about 15 times as active as the corresponding D-isomers.



NOREPINEPHRINE



EPINEPHRINE

Much of the hormones is oxidized to inactive compounds by the action of the enzyme monoamine oxidase (MAO) and others. The liver is the main site of this enzymic inactivation. They are also inactivated by methylation of the -OH in position-3.

Vanilmandelic acid (VMA) (which is 4-hydroxy-3-methoxy-mandelic acid) is one of the principal products excreted in urine. This metabolite accounts for over 60% of the catecholamine excretion in urine. The metabolites are conjugated with glucuronic acid or sulfate before excretion.

To exert physiological action, the hormones have to be bound to the tissues on which they act. This binding is inhibited by drugs like reserpine, cocaine and chlorpromazine which thus block adrenergic action. Others which structurally resemble the hormones (eg: β-hydroxy tyramine and alfa methyl-norepinephrine) compete for the binding sites in tissue and thus inhibit the hormonal action

Functions of norepinephrine and epinephrine: The general physiological effects are briefly summarized below:–

1. Norepinephrine exerts an overall vasoconstrictor effect without much effect on cardiac action. The blood pressure is increased.

Epinephrine causes vasodilatation of arterioles of muscle and vasoconstriction of arterioles of skin and splanchnic area besides increasing rate and force of contraction of the heart. The overall effect is a rise in blood pressure.

2. Epinephrine causes relaxation of smooth muscles of the stomach, intestine, bronchioles and urinary bladder and contraction of the sphincters of stomach and bladder. Its relaxant effect on bronchioles is used in the treatment of bronchial asthma.

3. *Metabolic effects:* Epinephrine is by far more potent than norepinephrine in metabolic functions:

(a) *Liver glycogenolysis:* The action of epinephrine is similar to that of glucagon. By stimulation of the liver enzyme, adenyl cyclase, there is a final increase of phosphorylase activity and glycogenolysis, causing an elevation of blood sugar level.

(b) *Muscle glycogenolysis:* Unlike glucagon, epinephrine enhances the cyclic AMP level in muscle also and causes glycogenolysis in it leading to increased blood pyruvate and lactate levels.

(c) Lipolysis is stimulated in adipose tissue leading to a rise in the nonesterified fatty acid (NEFA) levels of plasma.

4. Glucose uptake by tissues is diminished.

5. Insulin secretion by pancreas is decreased.

The above actions result in (1) increased output of glucose by liver glycogenolysis. (2) increased gluconeogenesis by liver from pyruvate and lactate and fatty acid (made available by epinephrine action on muscle and adipose tissue) also helping in increased output of glucose by liver and (3) diminished uptake of glucose by peripheral tissues. The glucose thus passing out into circulation from liver is readily and exclusively available to the nervous system to help in tiding over an emergency or stress.

Nature of adrenergic receptors: The receptors stimulated by these hormones are said to be of two types-the alfa and the beta adrenergic receptors. Norepinephrine mainly stimulates the alfa receptors and produces vasoconstriction. Epinephrine can stimulate both. Increased rate and force of contraction of heart, glycogenolysis and lipolysis are due to stimulation of the beta receptors which results in increased cyclic AMP production. On the pancreas, epinephrine stimulates mainly the alfa receptors leading to a depression of cyclic AMP level and a diminished insulin secretion.

Regulation of secretion of adrenal medulla: A number of factors like fear, anger and pain (emotional states), hypoglycemia, muscular activity and hypotension stimulate the secretion of the hormones. Hypotension mainly stimulates noradrenaline secretion while hypoglycemia promotes adrenaline secretion. The 'alarm reaction' is probably first triggered

off by adrenal medulla which pours out epinephrine into circulation which in turn stimulates ACTH secretion by the adenohypophysis. The latter may be an indirect effect mediated by a hypothalamic mechanism. Tumours of adrenal medulla (known as pheochromocytoma) produce intermittent hypertension, coronary insufficiency and cardiovascular failure. Estimation of catecholamines or vaniline mandelic acid (VMA) in urine helps in the diagnosis of the condition.

Disulfiram inhibits the enzymic synthesis of catecholamines from dopamine. Reserpine and guanethidine increase the destruction of catecholamines. They act therefore as antihypertensives and tranquilizers.

Tyramine, methylodpa and alfa-methyltyrosine, when administered, produce substances resembling catecholamines which compete with the hormone binding sites, and increase the circulating levels of catecholamines.

Phentolamine (Regitine) is a specific antagonist to noradrenaline. When injected intravenously it causes a rapid fall of blood pressure in 2 – 5 minutes. This can be used as a clinical test for pheochromocytoma.

THE ADRENAL CORTEX

This arises from the mesodermal tissue of the nephrotome portion of the embryo. It is essential for life. It elaborates several hormones, all of which are steroid derivatives. Kendall

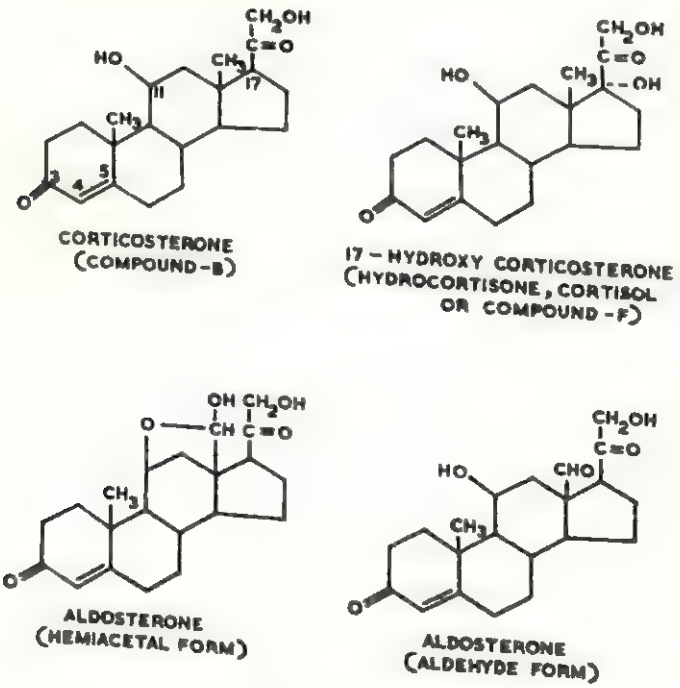


Fig. 28-1. Adrenal Cortical Hormones.

and associates isolated and studied a number of the hormones. They exert profound effects on (i) mineral metabolism, (ii) carbohydrate, fat and protein metabolisms (mainly carbohydrate) and (iii) sex hormone like actions. Nearly 50 distinct sterodial compounds are isolated from adrenal cortex and they are classified according to their principal actions into one of three groups. (a) mineralocorticoids, (b) glucoroticoids and (c) sex hormones. The sex hormones may be estrogens (18 carbon compounds), androgens (with 19 carbons) and progesterones (with 21 carbons). They resemble similar hormones produced by ovary and testis and are not further considered here. The mineralocorticoids and glucocorticoids have 21 carbons each.

Chemistry: The parent ring structure is the 'pregnane' ring. The active hormones have a double bond between C-4 and C-5, and two keto groups-one at position 3 and another at position 20. Certain key groups exert profound influence on the biological activity of the compounds. A 'OH' at position 21 enhances sodium retention and is necessary for the effect on carbohydrate metabolism. A hydroxy or ketonic group at position 11 is also necessary for the effect on carbohydrate metabolism but it diminishes the effect on mineral metabolism. A hydroxy group at position 17 enhances activity on carbohydrate metabolism.

The structures of the more important hormones are shown in fig. 28-1.

If in position 11 there is an 'O' instead of 'OH' it is 11-dehydrocorticosterone (compound A). If there is neither 'O' nor 'OH' it is 11-deoxycorticosterone.

Similarly 11-dehydro 17-hydroxy corticosterone (compound E or cortisone) and 11-deoxy, 17-hydroxy corticosterone (compound S). Replacement of the $-CH_3$ at position 13 by a -CHO (aldehyde group) gives rise to aldosterone, the potent mineralocorticoid. It can exist in the aldehyde form or the hemiacetal form.

The mechanism of action of the adrenal cortical hormones is at the level of the cell nucleus. They bind to specific receptor proteins in the cytosol and the steroid-receptor complexes enter the nucleus where they bind to specific sites on the chromatin transiently and influence mRNA synthesis and through that protein (enzyme) synthesis. Inhibitors of RNA synthesis will thus prevent action of these hormones.

Functions:

1. *Mineral metabolism:* They increase the reabsorption of sodium and chloride by the renal tubule and decrease their excretion in sweat, saliva, and gastro-intestinal secretions. Aldosterone is the most potent hormone in this regard. 11-Deoxy corticosterone and 11-deoxy, 17-hydroxy corticosterone (compound S) also have profound effects. They are produced in the zona glomerulosa. 11-Deoxycorticosterone acetate (DOCA) can be synthesized in the laboratory and can be absorbed by the buccal mucosa. Hence it can be administered sublingually as a substitute for aldosterone, though it is only 4% as active as aldosterone.

There is increased excretion of potassium (exchanged for the sodium being reabsorbed) and increased retention of sodium and water in the body.

Aldosterone secretion is not altered by the adrenocorticotropic hormone (ACTH). Low sodium intake in food causes an increase in production of aldosterone and vice versa. The regulatory mechanism seems to act through alteration of extracellular fluid volume, decrease of which stimulates aldosterone secretion which facilitates retention of sodium and an equivalent amount of water.

A specific aldosterone stimulating hormone (A.S.H.) is also said to function. Volume receptors said to be present in the walls of the renal afferent arterioles are stimulated by a decrease in the stretch of the arteriolar wall (fall in E.C.F. volume). This causes secretion of renin by the juxatglomerular cells which in turn causes secretion of 'angiotensin II'. The angiotensin II stimulates the relevant cells of the zona glomerulosa (of the adrenal cortex) to secrete aldosterone. Aldosterone secretion will lead to retention of sodium and water and expansion of ECF volume and thus causes stretching of the afferent arterioles of glomeruli and cuts down the secretion of renin. This is a 'feed back' mechanism of regulation.

Increased potassium concentration of plasma directly stimulates aldosterone production independent of ECF volume. The hormone seems to act at the nuclear level on the tubular epithelial cells.

Aldosteronism: In certain diseases like cirrhosis of the liver, nephrosis and cardiac failure, there is an increased production of aldosterone which causes retention of fluid in the body (edema and ascites). This is known as secondary aldosteronism. In primary aldosteronism there is a hyperfunction of adrenal cortex to produce more of aldosterone. Certain drugs (eg: Spiranolactone or Aldactone) block the action of aldosterone by binding to the aldosterone receptor and thus bring about diuresis and removal of excess water.

Licorice roots contain glycyrrhizinic acid which has mineralocorticoid activity. When it is used as a flavoring agent or in spices, there is a danger of causing hyperaldosteronism.

2. *Carbohydrate, lipid and protein metabolism:* Hormones with a 'O' or 'OH' at position 11 exert their effects mainly on carbohydrate metabolism and are called the glucocorticoids or the 'S' hormones. Corticosterone (compound-B), 11-dehydrocorticosterone (compound-A) and the 17-hydroxy derivatives of these (compounds E and F) belong to this group. They are produced mainly in the zona fasciculata and zona reticularis. They are insulin antagonists in many respects. They cause (1) increase in blood sugar level; (2) decrease the utilization of carbohydrate; (3) increase the synthesis of glycogen; (4) increase gluconeogenesis; (5) decrease lipogenesis and increase lipolysis; (6) decrease reabsorption of uric acid by renal tubules and thus produce an increased excretion of uric acid in urine (uricosuric effect); (7) cause involution of thymus and eosinopenia and lymphopenia and (9) increase gastric HCL and pepsin production by stomach and trypsin production by pancreas. (10) Stress: Glucocorticoids help to raise the blood pressure which falls in emotional or surgical shock.

The mechanisms of action for these diverse effects of the hormone are not clear. Their stimulation of gluconeogenesis seems to be on account of stimulation of the enzyme pyruvate

carboxylase. This and other enzymes concerned in gluconeogenesis and amino acid catabolism seem to be synthesized in larger amounts due to the stimulant action of the hormone on the cellular RNA synthesis in the liver.

Anti-inflammatory effects: Besides the above metabolic effects, the cortical hormones exert a profound anti-inflammatory effect, and are invaluable in the treatment of collagen diseases like rheumatoid arthritis. They also decrease the antigen-antibody response and are useful in treating allergic conditions. They probably suppress synthesis of nucleic acid and protein in the lymphocytes. Cortisol also depresses immune response in organ transplanatation procedures.

Synthetic analogues of steroid hormones: Minor alterations in the structure of the hormone molecule cause an accentuation of certain of the hormonal actions while suppressing most of the others. Steroids having profound anti-inflammatory effect with little or no metabolic effects are thus produced (eg: prednisone and prednisolone). Many of these compounds are used in therapy.

Regulation of secretion: ACTH regulates the secretion of glucocorticoids. Lowered cortisone levels in blood stimulate ACTH production.

Biosynthesis of the hormones: The steps in synthesis are similar to cholesterol synthesis and start with the simple 2-carbon molecule – acetyl coenzyme-A. The synthesis requires $NADPH+H^+$. Adrenal cortex is rich in ascorbic acid which has also probably a role in some of the reduction steps.

Pregnenolone is one of the important precursors formed. This can now follow the androgen pathway or the corticosteroid pathway. Progesterone is the first hormone formed from pregnenolone. 'Hydroxylases' capable of introducing an-OH group at 11, 17 or 21 positions are required in these pathways. Abnormalities in these enzymes are responsible for several defects in adrenal cortical function.

About 25 mg. cortisol and 2.5 mg. of corticosterone and less then 0.1 mg. of aldosterone are the principal hormones secreted by the adrenal cortex per day.

Fate of adrenal cortical hormones: The hormones are transported bound to an α globulin in the plasma (transcortin; corticosterone-binding-globulin; CBG). In the liver, they are reduced to their tetrahydro derivatives and then conjugated with glucuronic acid and excreted through bile. Some are reabsorbed (entero-hepatic circulation) and a portion enter systemic circulation to be excreted by the kidney. Thus liver is an important organ in inactivating the hormones. In hepatic failure, one cause for edema and sodium retention may be on account of prolonged action of these hormones due to failure of the liver to inactivate them.

Hyperfunction of adrenal cortex: Tumors of adrenal cortex produce hyperadrenocorticism. This manifests in (1) hyperglycemia and glycosuria; (2) retention of sodium and water resulting in edema and hypertension; (3) negative nitrogen balance; (4) hypokalemia and (5) hirsutism.

Hirsutism is on account of hyperaction of the androgens of the adrenal cortex. When this occurs in childhood, it is called adrenogenital syndrome. The female assumes male secondary sex characters (growth of beard and moustache) and males show excessive masculinization.

Cushing's syndrome: This may be primarily of pituitary origin (basophilic adenoma of pituitary). The increased ACTH stimulation will lead to a hyperfunctioning of the adrenal cortex. It may also occur due to a primary adenoma of the adrenal cortex.

The condition is characterized by obesity involving the face, neck and trunk (described as the buffalo type), purpura, hirsutism, hypertension, hyperglycemia and glycosuria.

Addison's disease: A hypofunction of adrenal cortex, usually due to a tuberculous infection, results in excessive loss of sodium and chloride in urine, low blood pressure, hypoglycemia, general weakness, wasting and a brownish pigmentation of the skin. The pigmentation is on account of excessive ACTH secretion by the pituitary, the ACTH having a melanocyte stimulating effect.

Diagnostic procedures:

1. Plasma levels of cortical hormones can be assayed using suitable laboratory methods. These will be elevated in Cushing's syndrome (normal 5 – 15 μg/100 ml.)

2. *Urinary excretion of the hormone derivatives.*

(a) *17-Hydroxycorticosteroids:* These are derived from the glucocorticoids and to a smaller extent mineralocorticoids. The hydroxy corticoids excreted in a day will be 4 – 10 mg. in females and 6 – 14 mg. in males. This is lowered in Addison's disease and increased in Cushing's syndrome.

(b) *17-Ketosteroids:* Phenolic and neutral ketosteroids are derived from estrogens and androgens respectively. The neutral 17-ketosteroids excreted in 24 hours urine are normally 5 – 15 mg. in females and 10 – 20 mg. in males. These are very much increased in adrenogenital syndrome, and decreased in Addison's disease.

THE THYROID GLAND

The thyroid gland secretes iodine-containing hormones which have a general stimulating effect on cell respiration and metabolism.

Biosynthesis and chemistry of the hormones: The thyroid has the ability to take up iodine from plasma against a concentration gradient and utilize it for hormone synthesis. About ⅓ of the inorganic iodine of plasma derived from food is taken up by the gland, and the rest is excreted mainly by kidney. Small amounts are also excreted in saliva, milk and gastrointestinal secretions. The iodine concentration in thyroid is ten to hundred times that in plasma. The TSH (thyroid stimulating hormone) of the pituitary stimulates the uptake of iodine by the gland. Thiocyanate and perchlorate competitively inhibit iodine uptake. Cyanide and dinitrophenol also inhibit the uptake by blocking cellular metabolism.

In the gland, the inorganic iodide (I^-) is oxidized to iodine (I or I^+) by a peroxidase enzyme with the loss of one or two electrons. The active iodine is now taken up by the tyrosine moiety of the glycoprotein, thyroglobulin, which is the characteristic protein of the colloid of the thyroid and has a molecular weight of 660,000. It has 115 tyrosine residues in its molecule. The sequence of iodination of tyrosine and the condensation of iodotyrosines to form the hormone are shown in figure 28-2.

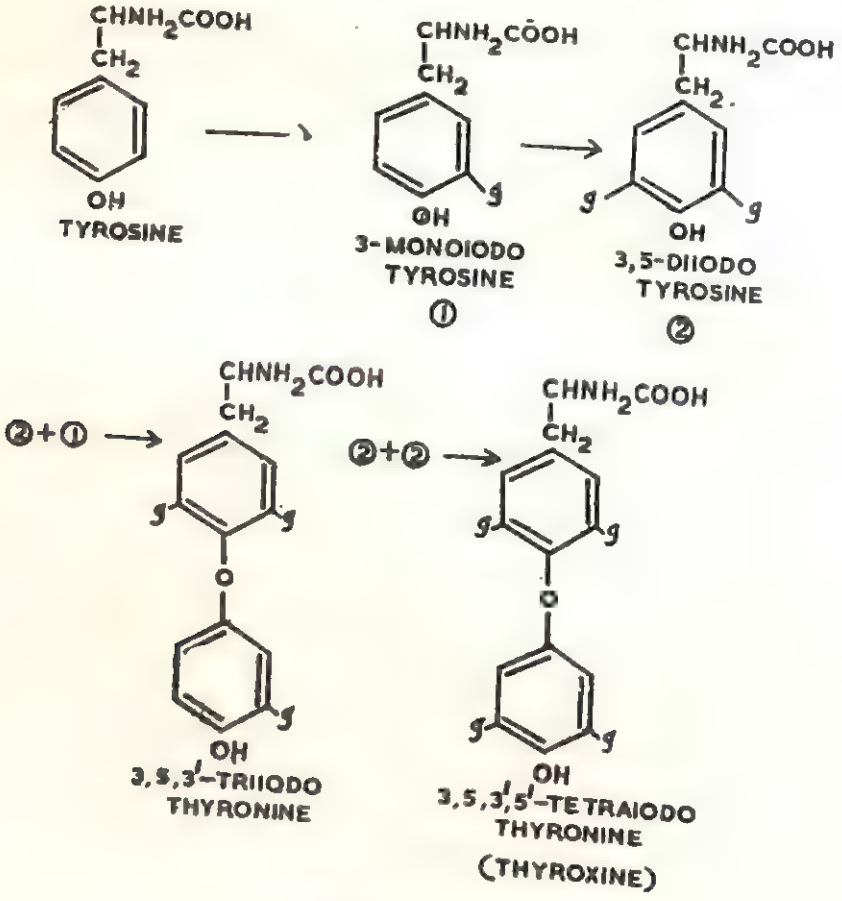


Fig. 28-2. Thyroid hormones.

The iodination of tyrosine and the subsequent coupling reaction between the iodotyrosines all occur while in the thyroglobulin molecule. The triiodo and tetraiodothyronine (T_3 and T_4) are then released by proteolytic enzymes present in lysosomes or vesicular membranes and enter the blood stream. Mono and diiodo tyrosine are also released, but are deiodinated and the iodine is again used. Release of the hormone from the thyroglobulin is accentuated by T.S.H. and by exposure to cold environment.

Transport in blood: Blood plasma contains two proteins – a thyroxine binding globulin (TBG) which migrates between α_1 and α_2 globulins on electrophoresis, and a thyroxine binding prealbumin fraction (TBPA) – which take up thyroxine and transport it. A trace amount of the hormone is also in the free state and probably represents the active form of the hormone. In a normal individual, the plasma thyroxine levels (measured as 'protein-bound-iodine' or PBI) vary from 4 – 8 μg/100 ml. But the T.B.G. has much reserve and can bind 3 times this amount.

T_3 may be the major thyroid hormone. It disappears from the blood 20 times faster than T_4. 80% of the circulating T_4 is converted to T_3 in the tissues, particularly in the liver and kidneys. The deiodination in the tissues may produce 3, 5, 3^1 triiodothyronine as well as 3, 5^1, 3^1 triiodothyronine. The latter is called reverse T_3 and is less active than T_3.

Inactivation of the hormones: In the tissues, the side chain is oxidized to form the tri or tetraiodothyroacetic acid (Triac and Tetrac) which are less active than the original compounds. They may further be deiodinated and excreted. The liver conjugates them with glucuronic acid or sulfuric acid and excretes them through the bile.

Factors regulating thyroid secretion: T.S.H. is required for the uptake of the plasma iodine by the gland, its oxidation and incorporation into the thyroxine and also for the release of the hormone from the gland. The hypothalamus also is said to elaborate a thyroglobulin releasing factor (TRF). Plasma levels of the thyroxine regulate the hormone production by enhancing or decreasing T.S.H. secretion of the pituitary. This is an example of feed back inhibition.

Functions of thyroid hormones:

1. *Calorigenic effect:* They increase the metabolism and oxygen consumption of all tissues.

2. *Protein metabolism:* In physiological levels, they are protein anabolic and are necessary for normal growth. In higher amounts, they cause excessive breakdown of protein due to an increased rate of metabolism and cause a negative nitrogen balance. There is increased excretion of urinary creatine.

3. *Carbohydrate metabolism:* The rate of absorption of glucose from the intestines is increased. This results in rapid hyperglycemia during an oral glucose tolerance test. But, since the rate of metabolism of glucose by tissues is also high, the level rapidly comes down to normal. There is also an increase in hepatic glucose-6-phosphatase activity and a more rapid insulin destruction. Glycogenolysis is increased in the liver and muscle due to increased sensitiveness to epinephrine. Gluconeogenesis is also enhanced. All these effects lead to the development of a diabetic state. But the utilization of glucose by tissues through glycolysis as well as citric acid cycle is not impaired. On the other hand it is increased. Hence the diabetes is not of a severe type.

4. *Lipid metabolism:* The hormones favour lipolysis in the adipose tissue and raise the plasma NEFA levels. This may be also an indirect effect through sensitization of the tissue to epinephrine action. The lipogenic effect of insulin is also enhanced. The important

diagnostic effect is on plasma cholesterol levels. In hyperfunction of the thyroid, there is an increased hepatic synthesis of cholesterol, but a greater increase in its oxidation to bile acids and elimination. The result is a decrease in plasma cholesterol levels.

In hypothyroidism, the reverse set of conditions act – a decreased synthesis but a greater decrease in oxidation leading to a rise in plasma cholesterol.

Mechanism of action:

The action of thyroid hormones is relatively slow. They enter the target cell and are bound to a specific carrier molecule which is directly associated with nuclear chromatin. In moderate concentrations, they have an anabolic effect. RNA content, amino acid transport into the cell and protein synthesis in the cell increase.

Higher concentrations of the hormones produce negative nitrogen balance. Protein synthesis is decreased; carbohydrate and lipid breakdown are increased; bone gets decalcified, mitochondrial swelling and uncoupling of oxidative phosphorylation occur.

Antithyroidal substances:

1. Thyroid hormone itself suppresses further secretion by the gland by a feedback mechanism. This action is mediated through T.S.H.

2. Deficiency of iodine in food and drink causes hyperplasia of thyroid (goitre). Excess of iodine causes hypofunction by decreasing T.S.H. output and is used in the treatment of hyperthyroidism.

3. Thiocyanate inhibits the uptake of iodine by the thyroid by blocking the concentrating mechanism.

4. Thiourea, thiouracil and sulfonamides inhibit synthesis of the thyroid hormone and produce goitre. The action may be by preventing oxidation of iodide directly by their action on iodide or through inhibition of oxidizing enzymes.

Hypothyroidism: In children, hypothyroidism manifests as cretinism. In adults it produces myxedema.

A cretin is dwarfish in size, has a thick tongue and thick skin, mentally retarded and sexually underdeveloped.

In myxedema, the skin is thick and puffy due to deposition of what is known as myxomatous material in subcutaneous layers: mental powers are dullened and there is hypersensitivity to cold.

The plasma cholesterol levels are elevated and B.M.R. decreased (– 20 to – 40%). Administration of thyroxine will remedy the condition. Each milligram of thyroxine can raise the B.M.R. by 2.8%.

Hyperthyroidism or exophthalmic goitre: The B.M.R. is increased from + 20 to + 80% or more. Wasting of the tissues and loss of weight are marked. There is hypersensitivity to hot climate. Body temperature, pulse and cardiac rate are increased. Plasma cholesterol level is decreased. Hyperglycemia and glycosuria may occur. Plasma P.B. I. levels are increased. The eye balls bulge out of the face (exopthalmos) and look large and protruding.

The thyroid activity can be evaluated by more recent techniques like determination of radioiodine uptake by the thyroid gland, estimation of T_3 by column chromatography and radioimmunoassay of T_4 and T_3.

In hyperthyroidism, a thyroid-stimulating protein substance other than TSH is found. It is not derived from the pituitary. Its site of origin is obscure. It duplicates TSH activity and exerts more prolonged activity than TSH. It is hence called 'long-acting thyroid stimulator-LATS.' Serum of hyperthyroid patients also contains a substance called LATS protector which prevents in vitro inactivation of LATS by thyroid tissue.

Thyrocalcitonin: Calcitonin was first extracted from parathyroids, but subsequently it was found that the thyroid contains even greater amounts of the hormone. It is produced by the C cells of the thyroid. It is a polypeptide with 32 amino acids and a molecular weight of 3,600. Injection of the hormone causes a rapid fall of serum calcium and phosphorous. Calcium passes from the blood into bone. Urinary excretion of calcium and phosphate remain unaltered. It does not alter the absorption or excretion of calcium and phosphorous by the intestines. The hormone exerts a direct effect on the bone - acceleration of calcium deposition and inhibition of its resorption. The hormone thus favours calcium retention by bone. Its secretion is increased in condition of hypercalcemia.

Calcitonin inhibits the synthesis of 1,25-dihydroxy-cholecalciferol in the kindey.

THE PARATHYROIDS

They are two pairs of glands embedded in the thyroid. They secrete a hormone called the parathyroid hormone or parathormone. It is synthesized as a preprohormone, which loses 25 amino acids to become the prohormone. The prohormone, in turn, loses 6 more amino acids to become the active parathormone. It has 84 amino acids, with a molecular weight of 8,500. Its amino acid composition had been worked out. Parathormone activates adenyl cyclase in bone and kidney. Urinary cyclic AMP is increased. The hormone exerts action chiefly on calcium and phosphorus metabolisms. Administration of the hormone (1) raises serum calcium: (2) lowers serum phosphorus and (3) increases urinary excretion of both calcium and phosphorus.

The increase in serum calcium is due to its mobilization from bone by increasing osteoclastic activity. The bone becomes decalcified. Increased excretion of phosphate due to diminished tubular reabsorption by proximal tubule and increased secretion by distal tubule leads to a lowering of serum inorganic phosphate. The plasma alkaline phosphatase activity is increased.

In addition to calcium loss, there is also a loss of mucopolysaccharide from bone matrix. Serum glycoprotien and sialic acid levels are increased following prolonged hormone action. The absorption of calcium and phosphorous from the gastrointestinal tract is increased. This effect is noticeable only if adequate amounts of vitamin D are present in diet.

The parathyroid hormone requires vitamin D for its function and is hence ineffective in rickets. It activates the vitamin D in the renal tissue by converting the 25-hydroxycholecalciferol to its 1,25-derivative. There is no storage of the hormone.

The secretion of parathormone is regulated by the ionic calcium levels of plasma.

Hypoparathyroidism: Usually this is caused by an accidental removal of the glands along with thyroid. Muscular weakness, lower serum calcium level and tetany are some of the manifestations.

Hyperparathyroidism: This is caused by tumours of the glands. Decalcification of bones, spontaneous fractures or bending of the bones, increased blood calcium level and decreased phosphate level are some of the symptoms. The large amount of circulating calcium may be deposited in the form of calculi in the kidney and pancreas. Abnormal calcification of pericardium and other soft-tissues may occur.

THE PITUITARY GLAND

The pituitary gland or hypophysis is composed of two parts – a neural component, neurohypophysis, and a buccal component, the adenohypophysis. The former consists of the posterior lobe and the infundibulum and the latter consists of the anterior lobe and the intermediate or middle lobe.

Hormones of the Anterior lobe:

Three types of cells are present in the gland: (i) eosinophilic cells which stain with acid dyes; (2) basophilic cells which stain with basic dyes; and (3) chromophobe cells or (neutrophils) which do not take either stain. The individual hormones formed by these are not clearly identified.

Growth Hormone (Somatotropin):

The growth hormone (GH) is a polypeptide with a molecular weight of 21,500. It has 191 amino acids and two disulphide bridges.

GH seems to act by stimulating protein synthesis, and by enhancing the uptake of amino acids by cells. It causes a nitrogen retention (positive nitrogen balance) and also a phosphorus retention. The transport of amino acids across the cell membrane is increased. This transport is independent of its subsequent utilization for protein synthesis.

The hormone stimulates lipolysis and increases plasma NEFA. Large doses may cause ketosis.

It inhibits uptake of glucose by the cells of extrahepatic tissues and the subsequent metabolism of glucose. Liver glycogen is increased due to increased gluconeogenesis. The combined effect of the two is hyperglycemia and a decreased glucose tolerance.

The effect on protein metabolism is similar to insulin action, but that on lipid and carbohydrate metabolisms is antagonistic to insulin action.

Calcium, sodium, potassium and phosphate are retained under the influence of the hormone.

Growth hormone stimulates the production of "somatomedins" (sulfation factors) from liver and kidney. They stimulate many of the anabolic effects of growth hormone and have also serum insulin like activity.

Somatostatin inhibits the release of not only growth hormone but also the release of insulin, glucagon, thyrotropin and FSH. It is used along with insulin, experimentally, in the control of severe diabetes mellitus to suppress the secretion of glucagon in particular. The D cells of the pancreatic islets and the gastric mucosa also secrete somatostatin.

Regulation of secretion of GH:

GH levels in plasma can be measured by immunoassay methods. They are highest in children and steadily decrease with age. The levels are same in both sexes or sometimes higher in females. Exercise and stress cause a transient increase in its levels. Hypoglycemia stimulates secretion of GH. Amino acids, particularly arginine, also cause an increased production of the hormone.

Glucose and glucocorticoids inhibit the secretion of GH.

The regulation of GH seems to be effected through the hypothalamus which is said to elaborate a specific 'growth hormone releasing factor' (G.H.R.F.) as well as a "growth hormone release inhibiting factor" (GH-RIH) or "somatostatin."

Hypersecretion of GH leads to the syndrome known as 'gigantism' if it occurs during growing period and 'acromegaly' if it occurs in the adult. Hyposecretion during growing periods produces dwarfism.

Lactogenic hormone: (Prolactin, mammotropin, luteotropic hormone, LTH). The hormone stimulates the grwoth of the mammary gland and activates the corpus luteum and stimulates the production of progesterone by that structure. It also stimulates growth like the GH. It is also a protein hormone and has a molecular weight of 23,400. It is inhibited by a hypothalamic "prolactin inhibiting factor."

The Tropic Hormones:

The anterior pituitary produces a number of hormones whose target organs are other endocrine glands whose function they stimulate. They are called the tropic hormones (trophein in Greek means 'to nourish'). The tropic hormones are produced inversely

in relation to the plasma concentration of the hormones of the target endocrines. Thus high levels of thyroxine in plasma will inhibit the production of thyrotropic hormone (thyroid stimulating hormone, T.S.H.) and vice-versa.

1. *Gonadotropins:* They are glycoproteins. (a) Follicle stimulating hormone (F.S.H.). It has a molecular weight of 30,000. It promotes the growth of the Graafian follicle in the female and the testicular growth and early stages of spermatogenesis in the male. The blood levels of F.S.H. are markedly increased during puberty and in the female at the time of ovulation. Its secretion is said to be regulated by the hypothalmus through a F.S.H. releasing factor.

(b) Luteinizing Hormone (LH) or Interstitial Cell Stimulating Hormone (ICSH): The hormone stimulates the final stages in the maturation of the Graafian follicle, ovulation and the development of corpus luteum in the female. It stimulates production of estrogen and progesterone. In male, it stimulates the interstitial cells to produce testosterone.

LH activates phosphorylase leading to glycogenolysis. The HMP pathway is enhanced and produces the $NADPH+H^+$ required for the synthesis of the steroidal hormones.

The secretion of LH is also regulated by the hypothalamus which produces a hypothalamic releasing factor (HRF).

The placenta of the pregnant female produces gonadotropic hormones similar to those of pituitary. They are called the chorionic gonadotropic hormones. The placenta is also said to elaborate a growth hormone and a thyroid stimulating hormone.

Pregnancy tests: The urine of a pregnant woman contains the chorionic gonadotropic hormones and this forms the basis of several tests for pregnancy.

Aschheim Zondek test: Injection of pregnant women's urine into immature female rats or mice causes hemorrhagic spots and yellowish corpora lutea in their ovaries.

Friedmann test: Injection of the urine into the ear vein of a virgin rabbit causes rupture and hemorrhage of the follicle in 24 hours. The test is also performed in different ways on the male frog, female toad and male toad. A precipitation test using antibodies to the chorionic gonadotropins is also used.

2. *Thyrotropic hormone (thyroid stimulating hormone, T.S.H.):* It is a mucoprotein with a molecular weight of 30,000. It increases the uptake of iodine by the thyroid, and its incorporation into the thyroid hormones. One mechanism of its action may be by stimulating glucose metabolism in the thyroid. The secretion of T.S.H. is regulated by a hypothalamic T.S.H. releasing factor (TRF).

3. *Adrenocorticotropic hormone (ACTH or corticotropin):* It is a polypeptide hormone with a molecular weight of 4566. A portion containing the active part of the molecule had been synthesized by Hoffman and associates in 1961.

It stimulates the synthesis and release of adrenal cortical hormones. The stimulus on the hormone synthesis acts at a point before pregnenolone formation. Since pregnenolone is the

precursor for mineralocorticoids, glucocorticoids, and also sex hormones, the synthesis of all the three is enhanced by ACTH. Aldosterone secretion is not much influenced by ACTH.

Injection of ACTH therefore produces all the effects of administration of corticosteriods on the carbohydrate, protein, lipid and mineral metabolisms. The action of ACTH seems to be by increased phosphorylase activity and increased HMP pathway. The glucose metabolism of adrenal cortex is enhanced.

Secretion of ACTH is reciprocally related to plasma levels of corticosteroids, Stress, fever, hypoglycemia, epinephrine, estrogens and vasopressin—all stimulate ACTH production. The hypothalamus is said to regulate ACTH secretion by producing a corticotropin releasing factor (C.R.F.). Three distinct factor α_1, α_2 and beta are described.

Abnormalities (hyper and hypofunction) of these tropic hormones produce changes similar to hyper-or hypofunctioning of the endocrine secretions which they influence.

Encephalins and Endorphins

The limbic system of the brain contains certain receptor sites which are capable of binding drugs like morphine and other analgesics (pain relievers). Small peptides capable of exerting morphine-like effects have been recently isolated from brain extracts. They bind to the same receptors in the limbic system and exert an analgesic effect. Two such peptides are isolated. Both are pentapeptides and differ only in the fifth c-terminal amino acid and are called 'encephalins.'

Leu-encephalin: Tyr-Gly-Gly-Phe-Leu.
Met-encephalin: Tyr-Gly-Gly-Phe-Met.

A beta-lipoprotein isolated from the anterior pituitary has 91 amino acids and the sequence of the amino acids 61 to 65 is same as Met-encephalin. Breakdown products of this beta-lipoprotein at this point have also analgesic properties. These small peptides are called 'endorphins.'

Hormones of the middle (or intermediate) lobe:

Intermedin or the melanocyte stimulating hormone (M.S.H.) is produced by the pars intermedia. Cortical hormones, epinephrine and norepinephrine inhibit MSH secretion. In Addison's disease, where cortical hormones are low, MSH is secreted in increasing amounts leading to brownish pigmentation of the skin.

MSH is said to exist in α, β and γ forms, each of which is a low molecular weight polypeptide.

Melatonin: It is a derivative of serotonin and its action is opposed to that of M.S.H. Melatonin lightens the color of the melanocytes of the skin. It is produced by the pineal body.

Hormones of the posterior lobe:

Two hormones are produced: (1) Vasopressin (pitressin); and (2) oxytocin (pitocin). The hormones are elaborated by the supraoptic neurons of the paraventricular nucleii of the

hypothalamus and are only stored in the posterior pituitary in association with two proteins – neurophysin I and II respectively. On release from the posterior pituitary they are carried in association with plasma proteins to the kidney, mammary gland and liver.

1. **Vasopressin:** It is synthesized in the cells of specialized neurons and combines with a large polypeptide called neurophysin and a small glycoprotein to form granules. The granules are discharged into circulation from the neurohypophysis in response to hypertonicity of the blood plasma or decrease in blood volume. They reach the distal convoluted tubules and bind to receptors on the tubular cells and cuase an activation of the adenylate cyclase and release of 3', 5'-cyclic AMP. This, in turn, causes the luminal membrane to become water permeable and leads to reabsorption of water by the distal convoluted tubules.

A deficiency of the hormone causes the syndrome known as 'diabetes insipidus.' Large volumes (as much as 30 litres/day) of urine of low specific gravity are passed.

2. *Oxytocin:* In causes contraction of the uterus and ejection of milk from the mammary gland. It is a cyclic polypeptide of 8 amino acids and has a molecular weight about 1,000.

SEX HORMONES

The male hormones:

Testosterone is the principal male hormone elaborated by the interstitial cells (Leydig

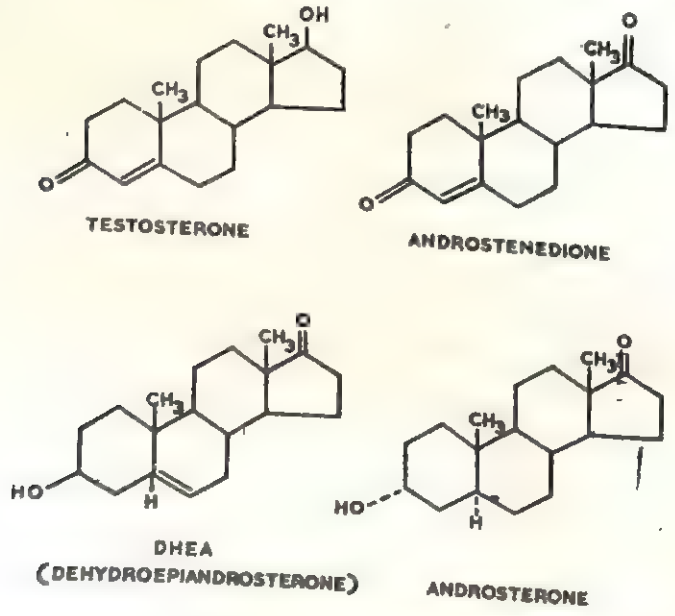


Fig. 28-3 Male Sex Hormones.

cells) of the testis. It is a 19 carbon steroid, the precursor of which is pregnenolone which is also the precursor for adrenal cortical hormones and of progesterone. Androstenedione and dehydroepiandrosterone (D.H.E.A.) are less potent hormones produced in the testis and adrenal cortex (see fig. 28-3 for their structure).

The hormones are transported by a specific plasma protein. Testosterone is metabolized to form androsterone and DHEA.

Testosterone promotes growth of secondary sex organs – epididymis, vas deferens, prostate, seminal vesicles and penis. It also promotes muscular and skeletal growth and is protein anabolic. There is retention of calcium and phosphate also. It exerts protein anabolic effect by increasing the RNA and RNA polymerase of the cell nucleus and aminoacyl transferase of the ribosome. It also stimulates the mitochondrial activity. The protein anabolic effect is useful in treatment of certain conditions of impaired growth or recovery from illness. Like adrenal cortical hormones, the androgens also first bind to cytosol receptor proteins, enter the cell nucleus and interact with nuclear chromatin to exert their actions.

Synthetic Analogues of Androgens:

Methyltestosterone with an additional methyl group attached to C-17 is active on oral administration. Derivatives without a methyl group at C-10 (called norsteroids-eg. 19 - nortestosterone and its 17-ethyl derivative – have potent protein anabolic effect and only a fraction of the androgenic effect. They are hence used as protein anabolic drugs.

The hormones are excreted in bile and urine after conjugation in the liver with sulfuric acid or glucuronic acid. Their derivatives account for a third of the urinary neutral 17 - ketosteroids.

The Female hormones:

The female hormones are divisible into two categories – (i) the follicular or estrogenic hormones and (ii) the progestational hormones. They are derived from the Graffian follicle and the corpus luteum respectively.

The follicular hormones: They are C-18 steroids. Ring A is aromatic and methyl group at C_{10} is absent. Ovary, placenta, adrenal cortex and testis can all form the hormones in different amounts.

Estradiol and estrone are the principal hormones. The excretory product in urine is estriol (see Fig. 28-4). Estrogens induce estrus in the lower animals. A vaginal smear will show the altered histological appearance of estrus (Allen-Doisy test in ovariectomized, sexually mature rats).

In woman, the hormones are concerned in the preparative phase of the menstrual cycle. They induce proliferation of endometrium, deepening of uterine glands, increased vascularity and changes in the fallopian tubules and vagina.

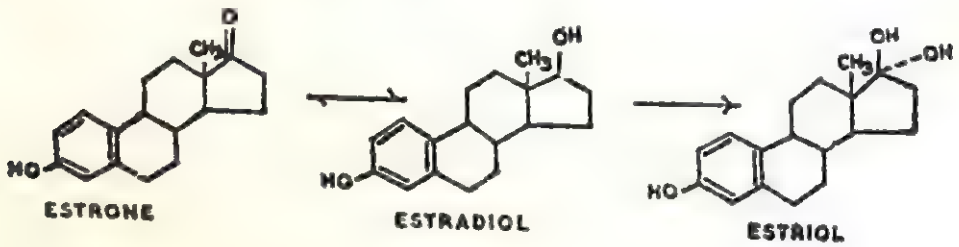
They suppress the secretion of FSH by pituitary. Estrogens are necessary for the maintenance of female secondary sex characters.

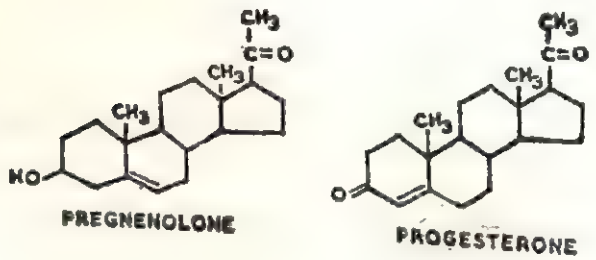
The hormones also act as cofactors in the transhydrogenation between the two pyridine nucleotides NADP and NAD

$$NADPH+NAD \xrightarrow[\text{Transhydrogenase.}]{} NADP+NADH.$$

Metabolic Action of Estrogens:

They exert protein anabolic effect locally on the target organ (uterus). The hormones are bound to a specific lipoprotein in that tissue. The RNA-polymerase activity of the tissue is increased. They also increase the phospholipid turnover rate in general and are hence lipotropic. They bring down the plasma lipids, if administered in conditions of hyperlipemia and coronary heart disease in men. Serum calcium and phosphate levels are increased on prolonged administration. Hypercalcification of bones may occur.





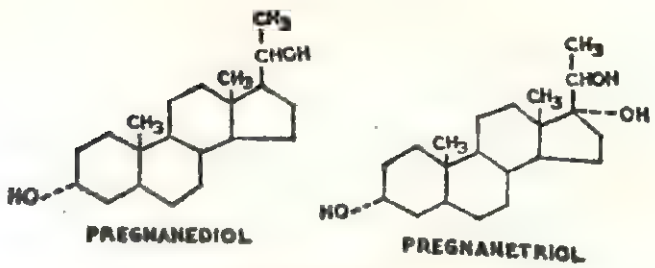


Fig. 28-4. Female Sex Hormones.

Synthetic hormones: Ethinyl estradiol and diethylstilbestrol are two synthetic substances which exert potent estrogenic effect when administered by mouth.

Progestation hormones or luteal hormones:

Progesterone is a C-21 compound and is synthesized from the common precursor, pregnenolone (See fig. 28-4).

The hormone is also formed by the placenta and also by adrenal cortex where it is the precursor of the several cortical hormones.

The action of the hormone is to cause endometrial development preparatory for the reception and nutrition of the embryo. It suppresses ovulation, estrus and the secretion of LH by pituitary. During pregnancy, progesterone production by corpus luteum continues through, till near term.

If pregnancy has not occured, the output of both estrogen and progesterone suddenly fall on or about the twenty-eighth day of the menstrual cycle, menstrual flow starts and the uterine endometrium starts sloughing.

Three-fourths of the progesterone is eliminated through bile through feces as pregnanediol and pregnanetriol and also through urine.

Progesterone:

Progesterone exerts an anti-ovulatory effect, if administered from 5th to 25th day of the menstrual cycle. It is however active only on parenteral administration. Synthetic progestins like 17-alfa-ethinyltestosterone and 17-alfa-ethinyl, 19-nortestosterone are active on oral administration, their activity being equal to or more potent than that of parenteral progesterone.

Relaxin is another hormone from the corpus luteum. It causes relaxation of symphisis pubis in animals. It is a polypetide hormone and is also produced by the placenta.

Synthetic hormones: Norethindrone and norethinodrel are two synthetic substances which are active when taken by mouth.

These and the synthetic estrogens form the basis for oral contraceptice therapy. By their use as prescribed, they suppress the secretion of pituitary F.S.H. and thus prevent ovulation from occurring. Thus pregnancy is avoided. A constant search for orally active compounds that suppress F.S.H. without exerting much hormonal effect is on.

References:

Antoniades, H.N. (ed): Hormones in Human Plasma; Nature and Transport., Little Brown, Boston; 1960.
Bayliss, R.I.S.: Synopsis of Endocrines and Metabolic Diseases., Williams & Wilkins; Baltimore, 1964.
Brown, J.H.U.: Basic Endocrinology., Davis, Philadelphia, 1962.
Chance, B., Estabrook, R.W. and Williamson, J.R. (ed): Control of Energy Metabolism, Acad., Pr., N.Y., 1965
Danowski, T.S.: Clinical Endocrinology. vols. 1-4, Williams and Wilkins, Baltimore, 1962.
Dorfman, R.I. and Ungar, F. (eds): Metabolism of Steroid Hormones; Acad., Pr., N.Y., 1964.
Eisensteine, A.B. (ed): The Biochemical Aspects of Hormone Action., Little Brown, Boston, 1964.
Gray, C.H. and Bacharach, A.L. (eds): Hormones in Blood, 2 vols., 2nd ed., Acad., Pr., N.Y., 1967.
Grollman, A: Clinical Endocrinology and Its Physiological Basis: Lippincot, Baltimore, 1964.
Harris, G.W. and Donovan, B.T. (eds): Pituitary Gland., Univ. Calif, Pr., Berkeley, 1966.
Karlson, P.(ed): Mechanism of Hormone Action, Acad. Pr., N.Y., 1965.
Krahl, M.E. (ed): The Action of Insulin on Cells. Acad. Pr., N.Y., 1961.
Martini, L. and Ganong, W.F. (ed): Neuroendocrinology, Vols. I and 2, Acad, Pr., N.Y., 1966-67.
McKerns, K.W. (ed): Functions of Adrenal Cortex; 2 vols., Appleton-Century-Crofts N.Y., 1968.
Pitt-Rivers, R. and Trotter, W.R. (eds): The Thyroid Gland., Butterworth, Washington (D.C.), 1964.
Tepperman, J.: Metabolic and Endrocrine Physiology, 2nd ed. Yearbook Med. Pub., Chicago, 1967.
Turner, C.D.: General Endocrinology, 4th ed., Saunders, Philadelphia, 1966.
Williams, R.H. (ed): Textbook of Endocrinology., Saunders, Philadelphia, 1968; Ind. Ed., Kothari, Bombay, 1970.
Wolstenholme, G.E.W. and O' Connor, C.M. (eds): Human Pituitary Hormones., Churchilf, Lond, 1960.
Young, F.G., Broom, W.A. and Wolff, F.W. (eds): Mechanism of Action of Insulin, Blackwell, Sci., Oxford; Eng., 1960.

29

FERTILITY AND ITS CONTROL

CONTROL of fertility, either by way of increasing it or decreasing it, is of interest not only from the medical point of view, but also from that of the sociologist and the economist. Decreasing human fertility successfully is a problem to be tackled on 'Top Priority' basis by developing countries like India so that the growth of the population does not exceed the growth of the limited resources. The following is a brief outline of the relevant chemical aspects of fertility and its control. For the sake of completeness, some of the more important physical and surgical methods are also mentioned.

Semen: The bulk of it consists of secretion from seminal vesicles and the prostate and serves as a vehicle for the spermatozoa produced by the testes. The semen, as ejaculated, is liquid in consistency, but rapidly sets to a gel. The coagulation is brought about by an enzyme 'vesiculase' produced by the prostate. The protein responsible for the coagulation process itself is secreted by the seminal vesicles. Zinc ions are required for the coagulation and prostate is one of the richest in zinc content. On standing for 15 to 20 minutes, the gel liquefies due to the action of another enzyme 'fibrolysin,' produced by the seminal vesicles. Semen also contains the enzyme 'acid phosphatase' elaborated by the prostate.

The pH of fresh human semen is about 7.1 to 7.3 and is maintained constant by means of buffers. On exposure to air, it loses CO_2 and becomes distinctly alkaline.

The spermatozoa can metabolize glucose, mannose and fructose with equal facility, but the seminal fluid contains mainly fructose which is secreted by the seminal vesicles by converting the circulating glucose to fructose. Seminal fructose levels fluctuate parallel to blood glucose levels. The secretion of fructose by seminal vesicles depends on the presence of circulating male hormones. The fructose content of semen decreases in castrated animals and is restored by injections of androgens. The fructose is metabolized anaerobically to pyruvic and lactic acids and aerobically to CO_2 and H_2O as in any other tissue. Human sperms lose their activity on standing for a few hours due to accumulation of lactic acid and H_2O_2. Human semen is devoid of catalase which can destroy peroxide as it is formed.

Semen also contains 'prostaglandins' produced by the seminal vesicles (and not by the prostate). They are synthesized from polyunsaturated fatty acids like linolenic and arachidonic. They have one or more hydroxy groups and some have also a keto group. The middle of the chain forms a cyclopentane ring with the remaining portions jutting out of it like two arms. The structures of two important members of this group prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$($PGF_{2\alpha}$) – are shown in fig 29-1 (A).

They stimulate smooth muscle, decrease blood pressure and increase heart rate. They antagonize the actions of catecholamines generally. The release of free fatty acids from adipose tissue is inhibited. On absorption from the vagina following coitus, they stimulate uterine contractions, which, in turn, favour the upward migration of the spermatozoa.

The volume of semen for a single ejaculation may vary from as little as 0.5 ml. to as much as 8.0 ml. and usually averages around 2.5 ml. It contains 25 to 400 millions of spermatozoa per ml. The spermatozoa are highly motile. Fertility is poor if the motility is less or the count is less than 60 million per ml. The heads of the spermatozoa should also be of uniform size.

The spermatozoa actively swim up into the uterus through the cervical plug of mucus. While they can survive for only two or three hours in the vagina, they can survive for about 40 hours in the uterus.

The Ovum: The ova are liberated periodically from the ovary, usually one for each menstrual cycle. The liberated ovum enters the fimbriated end of the Fallopian tube and travels along that tube to reach the uterine cavity in about 3 days after its liberation from the ovary. The movements of the cilia lining the tube as well as the peristaltic movements of the tube itself will aid in the propulsion of the ovum along the tube. These movements are under hormonal control.

Fertilization: Hyaluronidase enzyme is present in the seminal fluid and is actually concentrated on the surface of the spermatozoa. This enzyme, along with other less defined factors helps in the penetration of the 'corona radiata' the dense layer of granulosa cells around the ovum, by the spermatozoa in large numbers and one of the spermatoza will then fertilize the ovum. The fertilized egg remains free in the uterine cavity for about eight days before it gets embedded in the uterine wall (nidation or implantation) and is surrounded by a trophoblast from which placenta develops later.

Fertility Control and Family Planning:

Control of fertility in men: Administration of estrogens, progestin, 19-nortestosterone and other synthetic progestational steroids to man causes suppression of spermatogenesis. They seem to act directly on the testis, since the gonadotropin production of the pituitary is not altered. Spermatogenesis is restored after varying time lag on cessation of the hormones. Libido and potency also diminish during therapy. Hence the method is not applicable in fertility control.

Corticosteroids: Men suffering from Cushing's syndrome develop testicular atrophy. Administration of cortical hormones, even in large doses, to normal man does not alter the volume of the ejaculate, sperm count or seminal fructose content.

Nitrofuran derivatives, bis (dichloro-acetyl) amines and several other drugs also inhibit spermatogenesis during therapy. Injections of antibodies prepared against human testes and sperm also cause suppression of spermatogenesis.

Exposure of the testes to heat even for half an hour causes a rapid fall in sperm count.

Control of fertility in women:

1. *Inhibition of ovulation:* Ovulation time can be detected in women by (i) examination of vaginal smears daily for the appearance of cells with smooth pale cytoplasm instead of the heavily granulated, wrinkled, post-ovulatory cells. (ii) by alterations in the 'basal body temperature.' The temperature recorded in the mouth using a special thermometer graduated to the tenths of a degree Fahrenheit from 96 to 100 degress immediately on waking up in the morning and before getting out of bed is known as the basal body temperature (BBT). The fourteenth day of menstruation is most frequently the day when ovulation occurs. Rise from a relatively low to a relatively high BBT marks the ovulation time. During the follicular phase, estrogens exert a temperature (and metabolism) depressing effect followed after the mid-cycle by the thermogenic effect of progesterone.

On the analogy that ovulation does not occur during pregnancy, and since female sex hormones are increasingly elaborated during pregnancy, the effects of hormones on normal ovulation were studied. While estrogens as well as progestin can each inhibit ovulation, a combination of the two was found to be more effective and in smaller doses. It also had the advantage that controlled menstrual bleeding occurred during such therapy without ovulation. Several synthetic estrogen compounds and progestin compounds were tried and some of them proved efficacious on oral administration. Some act by depressing the production of FSH and LH of the pituitary, while others act directly on the ovary. Ovulation is prevented in either case.

In the sequential form of administration, estrogen alone is given for 14 or 15 days starting from the 5th day of menstrual cycle. This is followed by the combination of estrogen-progestagen for another 5 to 7 days and then discontinued for menstruation to occur. In the combination method, both estrogen and progestagen are administered together from the beginning i.e., 5th day till 24th day (total 20 pills) in every cycle. The menstruation that occurs during pill therapy is apparently no different from normal, but it is anovular. A modification of the pill, 'one-a-month' pill, contains a progestagen and a long acting oral estrogen, ethynylestradiol-3-cyclopentyl ether (quinestrol) and can be administered in a single dose late in the menstrual cycle (23rd to 25th day). The progestagen acts rapidly and induces bleeding. The estrogen is released slowly from depots and inhibits ovulation in the succeeding cycle.

Some progestagens (norethisterone and chlormadinone), in very small doses, alter the conditions of the cervix and the cervical mucus and probably the uterus and the Fallopian tubes and render the conditions sub-optimal for the migration of spermatozoa through the cervical canal and their survival in the uterus. They effectively bring about contraception without inhibiting ovulation.

The estrogens and progestagens most commonly used are listed in Table 29-1 and Figure 29-1 (B). It can be seen that the estrogens are mainly derived from estradiol while the progestagens are from 17_a-hydroxyprogesterone, 19-nortestosterone and testosterone.

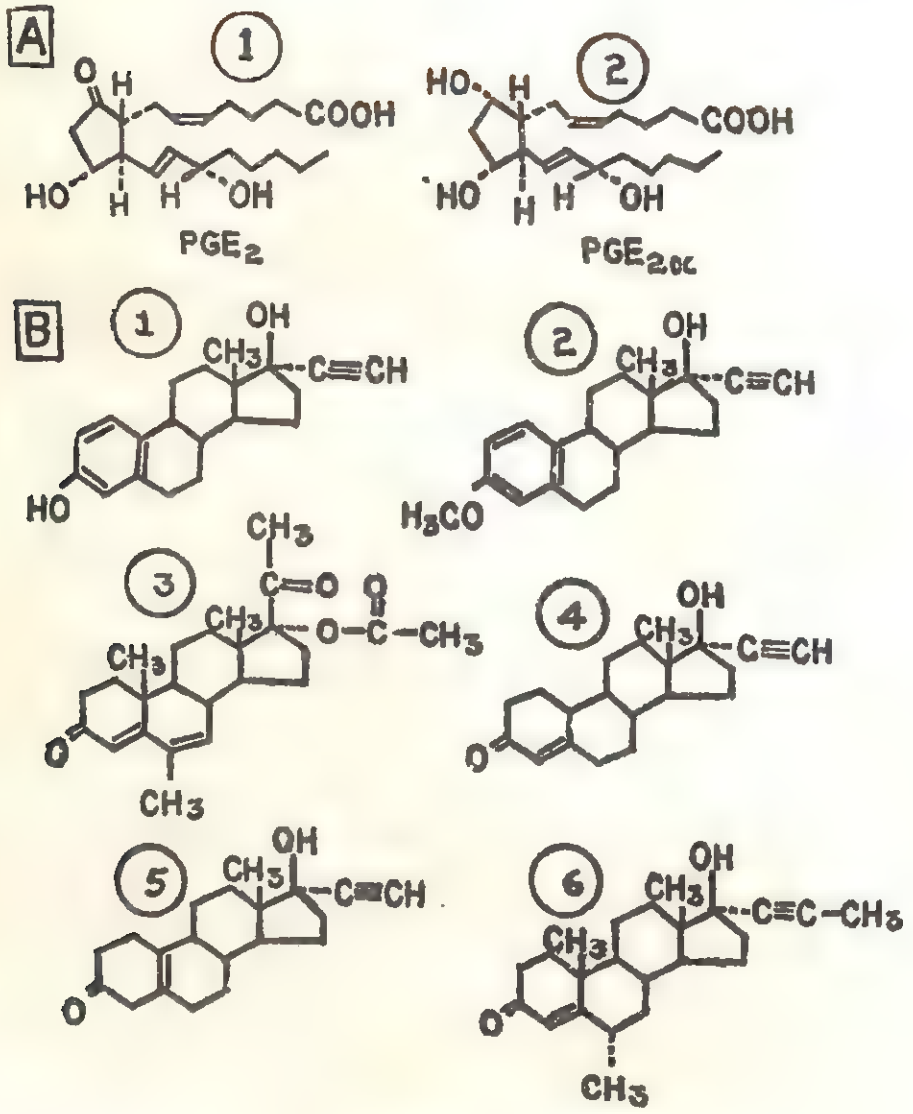


Fig. 29-1.

(A) (1) Prostaglandin E_2 (PGE_2)

(2) Prostaglandin $F_2\ \alpha$ ($PGF_2\alpha$)

(B) (1) Ethynylestradiol

(2) Mestranol

(3) Megestrol acetate (CL instead of CH_3 in position 6 will give chlormadinone acetate).

(4) Norethisterone (norethindrone)

(5) Norethynodrel.

(6) Dimethisterone.

TABLE 29-1.

Some of the hormonal derivatives used in oral contraception.

Estrogens (estradiol derivatives):
17 α-ethynylestradiol (estranol) (1).
17 α-ethynylestradiol, 3-methyl ether (mestranol) (2).

Progestagens:
17 α-hydroxyprogesterone derivatives:
Megesterol acetate (3).
Chlormadinone.

19-nortesosterone derivatives:.

17α-ethynyl-19-nortestosterone (norethisterone, norethindrone) (4).
17α-ethynyl-$\Delta^{5'10}$-estroanolone (norethynodrel) (5).
17α-acetate ester of norethindrone.
18α-methyl derivative of norethindrone (norgestrel).

Testosterone derivative:
Dimethisterone (6).

(The number in the brackets against the names of some derivatives indicates their structures numbered accordingly in fig. 29-1 (B).

Some non-steroidal compounds like dithiocarbomaxylhydrazine also exhibit inhibitory action on ovulation when administered orally. Several compounds are being tried which can be administered post-coitally and prevent implantation of the fertilized ovum. These are, however, only in experimental stages. The pill has been in use for several years in all parts of the world. A few toxic manifestations were observed such as diminished carbohydrate tolerance, diminished excretory function of the liver parenchyma and a tendency to thrombo-embolism, and phlebitis and varicose condition of peripheral veins.

2. *Safe period:* Ovulation occurs about the 14th day preceding the next menses (12 to 16th day). Taking two days as the maximal survival time of the spermatozoa in the uterine cavity after deposition in the female genital tract and one day as the maximal survival period for the ovum after liberation, in a woman having a regular 28 day menstrual cycle, the fertile period works out to be from 10th to 17th day after the previous menses. If the cycle is not regular, sutiable extra days have to be allowed on either side. In the normal cycle, days up to the 9th and from 18th till next menstrual period are infertile. Coitus during this period is safe from the danger of pregnancy.

3. *Local methods:* Both mechanical and chemical appliances can be used locally to either prevent access of spermatozoa to the ovum or to make the spermatozoa nonviable. Usually, both methods are used simultaneously in combination (one mechanical and one chemical) to get more reliable control.

Mechanical: (i) Diaphragm: (ii) cervical cap: (iii) condom. The first two fit against the cervix and cause mechanical obstruction to the upward migration of the spermatozoa. The condom is worn by the male.

Chemical: Jels, creams and aerosol foam tablets are applied by suitable applicators close to the cervix in the vagina a short time before intercourse, and allowed to remain for several hours thereafter. They contain potent spermicides which inactivate the spermatozoa from the ejaculate.

4. *Coitus Interruptus:* Here the male withdraws before ejaculation occurs. Spermatozoal contamination of the urethral passages and secretion of the Cowper's gland make this an unreliable method.

5. *Surgical Methods:* 1. Sterilization in the male: Vasectomy is the operation done and involves removal of a portion of the vas deferens on either side and ligating the cut ends. The spermatozoa do not have an exit from the testes and in course of time the portion of the testes concerned with spermatogenesis atrophies, but the endocrine portion remains in tact. In a properly done operation, 100% contraception is achieved. Surgical methods for the reconstruction of the vas are available, if required at a later date. 2. Sterliziation in the female: This is also a 100% successful procedure. Portions of the Fallopian tube are removed and the stumps ligated to prevent the entry of ovum into the uterine cavity. Reconstruction of the tubes is possible, if required, at a later day.

6. *Intrauterine Contraceptive Device (IUCD Loop):* A coil or loop of an inert plastic material is introduced with the aid of an applicator through the cervical canal into uterine cavity and allowed to remain there. It can remain for years in uncomplicated cases. By its mere presence in the uterine cavity, conception is prevented. This may be on account of foreign body reaction in the endometrium making it unsuitable for nidation (implantation) to occur or other unknown causes such as alteration in pH of the intrauterine fluid, changes in the uterine and tubal movements etc. The intrauterine fluid volume is decreased, viscosity increased, urea, albumin, lipid and osmotic pressure are increased.

7. *Further Studies:* Antizygotic agents like antiestrogen (MER-25) inhibit the development of ovum in birds. Antispermatogenic agents like nitrofurans and dinitropyrroles, orally administered, cause inhibition of spermatogenesis and infertility in experimental animals. Immunological methods like injections of testicular extracts and sperm antibodies may also be developed into practicable methods of the future. Parenteral administration of cadmium salts causes degenerative necrosis of the testis and ovary in animals. While the ovarian degeneration is reversible, testicular changes are not reversible. Zinc and selenium exert a protective action against the cadmium damage.

Some of these methods may, in the near future, become applicable to the human after further development and modifications.

8. *Abortion:* Abortion has been legalized in some countries for use under certain conditions. But it is almost universally practised in all countries furtively to get rid of unwanted pregnancies. In addition to several surgical and medical procedures available, a recent development is the use of protaglandins for inducing abortion. PGE_2 and $PGF_2\alpha$ are under extensive clinical trials in several countries including India. Intravenous, vaginal, intrauterine and intraamniotic routes of administration induced abortion at any stage of pregnancy including the first few days after missing the period. The mode of action appears to be by inducing rhythmic uterine contractions. Intraamniotic method is possible only after the 15th week of pregnancy, but the other routes of administration can be used at all stages of pregnancy. Toxic side effects like vomiting, diarrhoea and broncho-constriction may occur in a few instances.

In our country, the most popular methods are the use of IUCD or loop, the condom (popularized under the name 'Nirodh'), the pill and surgical operations of vasectomy in the male and tubectomy in the female. They are available to the public at practically no cost as they are heavily subsidized by government. In fact monetary inducements are also offered to those who avail some of these facilities.

References:

Bergstrom, S. Green, K. and Samuelsson, B.: Prostaglandins in Fertility Control., Third Conference on 'prostaglandins in Fertility Control., W.H.O. Res. and Trg. Centre on Human Reproduction Karolinska Inst., Stockholm, Sweden, 1972.

Calderone Mary, S.: Mannual of Contraceptive Practice, Williams and Wilkins, Baltimore, 1964.

Hartman, Carl, G.: Science and the Safe Period., Williams and Wilkins, Baltimore, 1962.

Lednicer, D.: Contraception – the Chemical Control of Fertility., Marcel Dekker Inc., N.Y., 1969.

Pincus, G.: The Control of Fertility, Acad. Pr., N.Y. 1965.

Roy, Somnath and Das, Rabi Prosad: Reproductive Bilogy., proceedings of the 1st Seminar, Central Family Planning Inst., and Ind., Council of Med. Res., New Delhi, 1968.

30

ANTIMETABOLITES

CERTAIN substances are capable of inhibiting the normal metabolism of a metabolite by their structural resemblance to the metabolite. These are called 'anti-metabolites' and the phenomenon is described as 'metabolic antagonism.' Several examples were already cited in the course of the preceding chapters. An attempt is made here to consolidate the information. Recent advances in antibiotics, anti-cancer and anti-viral drugs and drugs acting on the nervous system are all based on metabolic antagonism.

Metabolic antagonism is mainly on account of the competitive inhibition of enzymes exerted by compounds structurally similar to the substrate. Instead of the substrate combining with the enzyme, the antagonist combines with the enzyme. The combination is irreversible since no products can be formed from the antagonist. Such an inhibition can be overcome in the presence of excess of substrate which can displace the antagonist in most cases, e.g., inhibition of succinic dehydrogenase by malonate. There are however a few instances where even this is not possible. In such cases the affinity of the antagonist to enzyme is even stronger than that of substrate to enzyme.

Certain other antimetabolites combine with the enzyme, not at the active site where substrate combines, but at some other point in the enzyme, and exert an effect of decreasing enzyme activity. This inhibition is not reversed by an excess of substrate since the two occupy different sites on the enzyme.

A third set of antimetabolites not only combine with the active site of enzyme instead of the substrate, they are even converted into products by the enzyme, e.g., fluoroacetate can be incorporated by the condensing enzyme. But the fluorocitrate formed is not acted upon by the enzyme 'aconitase' with the result the further utilization of the compound is blocked at the level of a subsequent enzyme.

Practical applications of metabolic antagonism:

1. *Sulfonamide drugs:* Folic acid is a vitamin for the higher animals including man. The micro-organisms can however synthesize folic acid from para amino-benzoic acid (PABA) and for them PABA is the vitamin. Sulfonamide group of drugs, by their structural resemblance to PABA cause competitive inhibition of the enzymes concerned in this synthesis and thus prevent growth and reproduction of micro-organisms.

2. *Antibiotics:* These are substances produced by fungi and micro-organisms which are detrimental to the growth of other micro-organisms.

Penicillin: This was discovered by Fleming in 1929 and pursued by Florey and others. A substance produced from the fungus 'penicillium' is antibacterial on account of its action on the bacterial cell wall where it inhibits the incorporation of N-acetyl-neuramic acid into the polymerized mucopeptide structure of cell wall.

Streptomycin: This is produced by the organism 'streptomyces griseus' and inhibits gram negative bacteria. It seems to exert competitive inhibition of the enzymes of the ribosome and inhibit incorporation of certain amino acids like phenyl alanine while stimulating the incorporation of certain other amino acids like leucine with the result an abnormal protein is synthesized by the ribosomes. This protein cannot be utilized by the organism.

Tetracyclines: Aureomycin, terramycin and achromycin are some of the drugs belonging to this group. These are produced by organisms of the type of 'streptomyces aureofaciens.' They act by inhibiting protein synthesis by causing synthesis of an abnormal R.N.A.

Chloramphenicol: This is formed by 'streptomyces venezuelae.' It is particularly active against rickettsial and gram negative infections. It acts by immobilizing the function of messenger R.N.A. by competing with it for active sites on the ribosome.

Peptide antibioties: Small peptide molecules produced by some micro-organism like 'bacillus brevis' act as antibiotics e.g., tyrothricin and gramicidin. Bacitracin is produced by B-subtilis. All these act as antibiotics.

Acitnomycin is another bacteriostatic and tumour-inhibiting antibiotic. It is also a small peptide. It binds to the DNA and causes inhibition of the DNA directed RNA synthesis.

Puromycin: It is an antibiotic which is highly toxic to the host organism as well. Hence it is mainly used in the study of protein synthesis in the laboratory. It has a structural resemblance to the aminoacyl-sRNA complex and gets incorporated into the polypeptide chain being synthesized and causes the incomplete polypeptide chain to be released from the ribosomal template.

Chemotherapy against Cancer: Folic acid antagonists – aminopterin, methotrexate, and amethopterin – are used in treatment of leukemias and in metastatic choriocarcinoma in women. They act by inhibiting the one-carbon transfer required in the synthesis of histidine, purine and pyrimidine compounds. They seem to act mainly by inhibiting the enzyme folic acid reductase which converts the folic acid to the active tetrahydro form.

Pyrimidine compounds with fluorine substituted in position 5 (e.g. 5 – Fluorouracil, FU) can be used in treatment of cancer. They get incorporated into nucleic acid like the natural pyrimidine compounds and block further function of the nucleic acid formed. Azaserine (- OH group of serine is substituted by azide group) and 6-mercaptopurine (– SH group replacing amino group of adenine in position 6) act similarly.

In addition to substituents of the pyrimidine and purine rings, ribose can be substituted by arabinose. Cytarabine (arabinosyl cytosine) and vidarabine (arabinosyl adenine) are used in the chemotherapy of cancer and in viral infections.

Azathioprine is catabolized to 6-mercaptopurine and is used in organotransplants to supress immunological rejection of the transplant.

5-Iododeoxyuridine is used in the local treatment of herpetic keratitis (caused by the herpes virus).

Methotrexate: The conversion of UMP to TMP (uridylic acid to thymidylic acid) involves the oxidation of tetrahydrofolate to dihydrofolate. For this reaction to continue smoothly, the dihydrofolate has to be reconverted to tetrahydrofolate by a reductase enzyme (*dihydrofolate reductase*). This enzyme is inhibited by methotrexate (amethopterin). It is used as an anticancer drug.

Insecticides and herbicides: D.D.T. inhibits oxidative phosphorylation in the mitochondria of the housefly and other insects.

Chlordan and gammexane act similarly.

Organic phosphates: Tetraethyl pyrophosphate (TEPP) and other such substances present in nerve gases act by inhibiting the cholinesterase. Diisopropyl fluorophosphate (DFP) is another similar compound.

Disulfiram: (Antabuse or tetraethylthiuram disulphide) is used in alcoholics. It inhibits the utilization of acetaldehyde formed from ethyl alcohol. The accumulation of acetaldehyde leads to several unpleasant symptoms and sensations in the alcoholic with the result one gives up alcohol.

I.N.H. (Isonicotinic acid hydrazide), the antituberculous drug, is also an antimetabolite inhibiting the oxidative enzymes of the mycobacteria.

Antihistamines: Their mode of action is not clear, but they antagonize the physiological action of histamine.

Tranquilizers: They also act as antimetabolites in some phases of the metabolism of C.N.S.

References:

Busch, H. and Lane, M.: Chemotherapy., Yearbook Med. Pub., Chicago, 1967.
Hochster, R.M. and Quastel, J.H. (eds.): Metabolic Inhibitors, vols. 1 and 2., Acad., Pr., N.Y., 1963.
Martin, G.J.: Biological Antagonism., Blackiston, Divn. McGraw-Hill, N.Y., 1961.
Northey, B.H.: Sulphonamides and Allied Compounds, Reinhold, N.Y., 1948.
Webb, J.L.: Enzymes and Metabolic Inhibitors, Vols. 1 and 2. Acad. Pr., N.Y., 1963 and 1966.
Wooley, D.W : A Study of Antimetabolites, Wiley, N.Y., 1952.

RECOMMENDED ADDITIONAL READING

Bondy, P.K. and Rosenberg, L.E.: Duncan's Diseases of Metabolism, Vols. 1 and 2, 7th ed., Saunders, Philadelphia, 1974.

Cantarow, Abraham and Schepartz, Bernard; Biochemistry, 4th ed., Saunders, Philadelphia, (Internatl. Students' ed., Toppan, Tokyo, 1967).

Conn, E.E. and Stumpf, P.K.: Outlines of Biochemistry, 2nd ed., Wiley Eastern, New Delhi, 1969.

Fruton, J.S. and Simmonds, S.: General Biochemistry, 2nd ed., Wiley, N.Y., 1961.

Greenberg, David, M. (ed): Metabolic Pathways, Vols. I, II, III and IV. Acad. Pr., N.Y., 1960, 61, 69, 70.

Karlson, P.: Introduction to Modern Biochemistry translated by Doering, H., Acad. Pr., N.Y., 1975.

Latner, A.L.: Cantarow and Trumper Clinical Biochemistry, 7th ed., Saunders, Philadelphia, 1975.

Lehninger, A.L.: Biochemistry, 2nd ed., Worth Pub. Inc., N.Y., 1975.

Martin, D.W., Mayes, P.A. and Rodwell, V.W.: Harper's Review of Biochemistry, 19th ed., Lange-Maruzen, 1983.

Mazur, A. and Harrow, B.: Textbook of Biochemistry, 10th ed., Saunders, Philadelphia, 1971.

McGilvery Goldstein: Biochemistry–A Functional Approach, 3rd. ed., Igaku-Shoin/Saunders, 1983

Orten, J.M. and Neuhaus, O.W.: Human Biochemistry, 15th ed., Mosby. St. Louis, Missouri, 1975.

Passmore, R. and Robson, J.S. (Eds.): A Companion to Medical Studies, Vol. 1, 2nd ed., Blackwell Sci., Pub., Oxford, London, etc.; 1976.

Pritham, G.H.: Anderson's Essentials of Biochemistry, Mosby., St. Louis, Missouri, 1968.

Smith, E.L., Hill, R.L., Lehman, I.R., Lefkowitz, R.Z., Handler, P. and White, A.: Principles of Biochemistry, 7th. ed., McGraw-Hill International, 1983.

Stryer, Lubert: Biochemistry, Freeman, San Francisco-Toppan, Tokyo, 1975.

Suttie. John. W.: Introduction to Biochemistry, Holt Rinehart and Winston, N.Y., 1977.

Thorpe, W.V., Bray, H.G. and James, S.P.: Biochemistry for Medical Students, 9th ed., Churchill, Lond., 1970.

West, E.S., Todd, W.R., Mason, H.S. and Van Bruggen, J.T.: Textbook of Biochemistry, 4th ed., Macmillan, N.Y., 1966.

Yudkin, M. and Offord, K.: Comprehensible Biochemistry, Longman (England), 1973.

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